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Preface

The 2nd International Conference on Modern Medicine and Global Health (ICMMGH 2024) is an annual conference focusing on research areas including Biomedical Engineering, Pharmaceutical Science, Medicine, and Public Health. It aims to establish a broad and interdisciplinary platform for experts, researchers, and students worldwide to present, exchange, and discuss the latest advance and development in Biomedical Engineering, Pharmaceutical Science, Medicine, and Public Health.

This volume contains the papers of the 2nd International Conference on Modern Medicine and Global Health (ICMMGH 2024). Each of these papers has gained a comprehensive review by the editorial team and professional reviewers. Each paper has been examined and evaluated for its theme, structure, method, content, language, and format.

Cooperating with prestigious universities, ICMMGH 2024 organized three workshops in Kuala Lumpur, Guildford and Wuhan. Professor Mohammed J.K. Bashir chaired the workshop “Assessment and Disinfection of Pathogenic Microorganisms in Water Supply Systems”, which was held at Universiti Tunku Abdul Rahman. Dr. Roman Bauer chaired the workshop “Computational Analysis and Modeling for Biomedicine”, which was held at University of Surrey. Dr. Shuai Chen chaired the workshop “Dietary Nutrient Bioactive Compounds and Chronic Diseases”, which was held at Wuhan University.

Besides these workshops, ICMMGH 2024 also held an online session. Eminent professors from top universities worldwide were invited to deliver keynote speeches in this online session, including Professor Mohammed J.K. Bashir from Universiti Tunku Abdul Rahman, Dr. Omda Omran from University of Central Florida. They have given keynote speeches on related topics of Biomedical Engineering, Pharmaceutical Science, Medicine, and Public Health.

On behalf of the committee, we would like to give sincere gratitude to all authors and speakers who have made their contributions to ICMMGH 2024, editors and reviewers who have guaranteed the quality of papers with their expertise, and the committee members who have devoted themselves to the success of ICMMGH 2024.

Professor Mohammed J.K. Bashir
General Chair of Conference Committee

Workshop

Workshop – Kuala Lumpur: Assessment and Disinfection of Pathogenic Microorganisms in Water Supply Systems

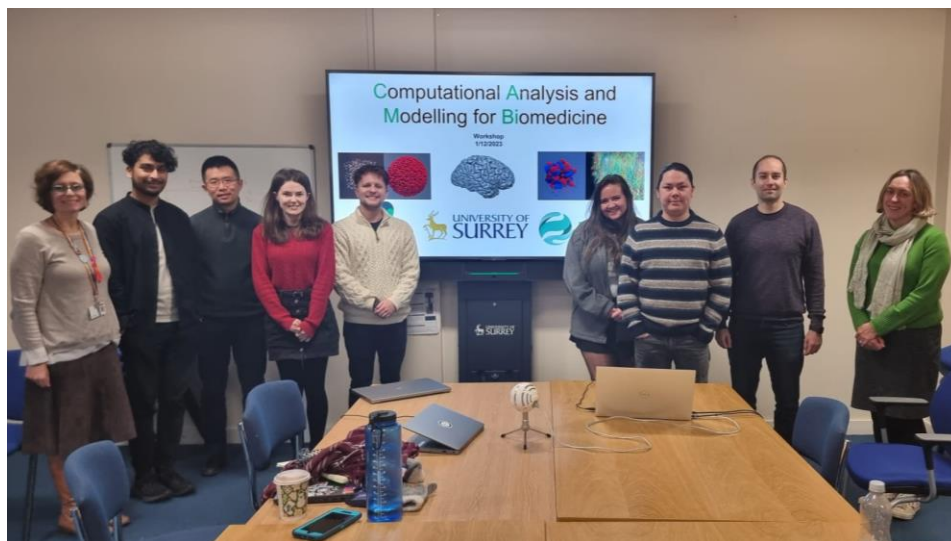


January 5th, 2024 (UTC+8)

Department of Environmental Engineering, Universiti Tunku Abdul Rahman

Workshop Chair: Professor Mohammed J.K. Bashir, Professor in Universiti Tunku Abdul Rahman

Workshop – Guildford: Computational Analysis and Modeling for Biomedicine



December 1st, 2023 (GMT)

Department of Computer Science, University of Surrey

Workshop Chair: Dr. Roman Bauer, Senior Lecturer in University of Surrey

Workshop – Wuhan: Dietary Nutrient Bioactive Compounds and Chronic Diseases



December 2nd, 2023 (UTC+8)

Wuhan University Food Nutrition Research Center, Wuhan University

Workshop Chair: Dr. Shuai Chen, Associate Professor in Wuhan University

The 2nd International Conference on Modern Medicine and Global Health

ICMMGH 2024

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Correlation and related impact of migratory routes of seasonal birds in India and Japanese encephalitis (JE) epidemic regions in India

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Abstract. Japanese encephalitis (JE) which is also called epidemic encephalitis B, is able to cause severe inflammation in human body especially the central nervous system (CNS). Base on the fact that birds are able to transmit virus as same as pigs in the local regions in India, here will be more discussions and analyses about the correlation and related impact of migratory routes of seasonal birds in India and JE epidemic regions in India base on the case studies in the upcoming articles. Additionally, to figure out the solution of the problem of the continuously JE epidemic over the whole country. Throughout the process, a tight relation between wild birds and JEV was discovered from the previous researches, which also reflected the tendency of JE endemic in regions in India affected by the distribution of bird sanctuaries. Based on policies established for other epidemic prevention before in other countries, a few suggestions were listed to give a supportive reference for potential JE epidemics. This can either enhance the security of citizens living in the endemic area and raise the awareness to wildlife around them.

Keywords: Japanese encephalitis (JE), seasonal migratory bird, reservoir host, JE SA-14-14-2 vaccine

1. Introduction

Epidemic encephalitis B is a type of epidemic infection that mainly causes by the epidemic encephalitis virus. The virus has 5 genotype, type I mainly exist in the eastern Asia whereas type III mainly exist in the southern Asia. The pathogen of epidemic encephalitis virus was isolated first time from Japanese researchers in 1934 thus it is also called Japanese encephalitis (JE) [1,2]. The clinical feature can be high fever, hyperspasmia, headache, central respiratory feature [1]. Since the first clinical case discovered in 1950 in India, more JE cases begun to be reported in southern, western and eastern states and the epidemic started spreading to the northern India (figure 1 and figure 2). Uttar Pradesh experienced the most severe JE in history in 2005, with 3867 confirmed cases and 1315 death that the mortality rate is roughly 34%. In some regions of the southern India the infection percentage is 70% which is even higher, and the majority of the patients are children between 5–9-year-old.

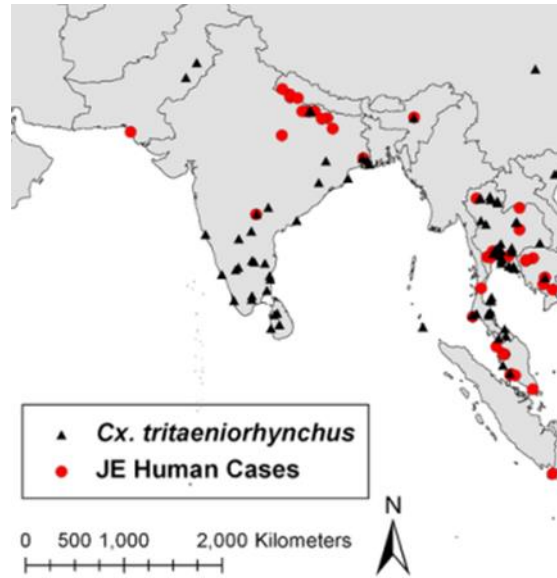


Figure 1. JE cases were reported in India.

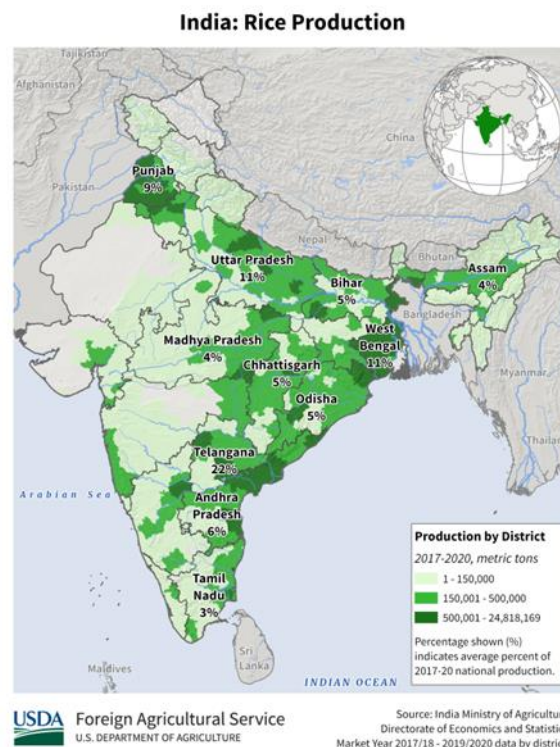


Figure 2. The rice planting land distribution in India [3].

As a threatening disease, JE is mainly transmitted by *Culex tritaeniorhynchus* which commonly reproducing in the lands planting rice and pounds etc. [3]. The tight relation between the rice planting land distribution and the population of *Culex tritaeniorhynchus* is available in the figure 2 and figure 3. There are numbers of *Culex tritaeniorhynchus* produced every day during the breeding season thus the residents living around have the highest possibility to be bitten by infected *Culex tritaeniorhynchus* [4].

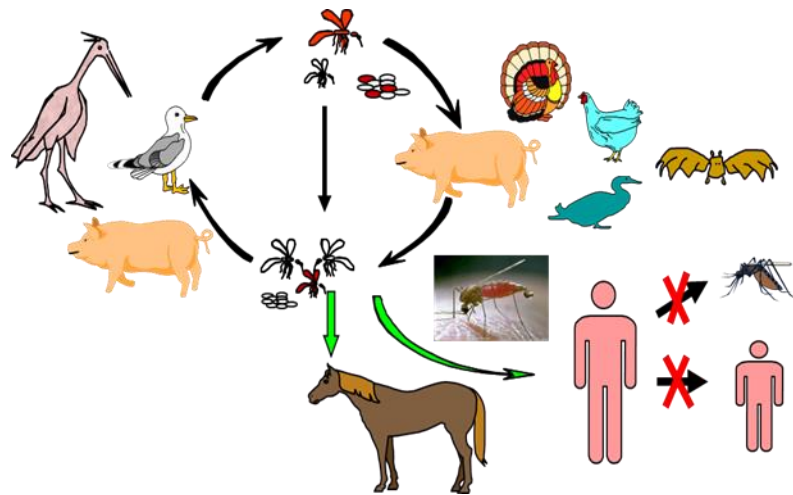


Figure 3. Diagram of *Culex triaeniorhynchus* transmitting JE [5].

Culex triaeniorhynchus are unable to pass the virus through generations that intermediates are required to preserve the virus and transmit back to them again which also enabled them to infect human [2]. Therefore, JEV can widen the epidemic range and causing longer impacts. In epidemic seasons or epidemic areas, the JE will widely spread in the piggeries previously before the virus infecting human, which shows that pigs are an essential factor of JE [2]. Though it was confirmed that pigs are the main factor to deliver the JEV, the seasonal migratory birds are more likely to be the object that transmit virus over regions or countries. Otherwise, there is no appropriate reason to explain the tendency shows on the diagram of JE epidemic distribution below, that the lands planting rice, piggeries and population distribution in India roughly divided apart which means JEV can merely transmit JEV from the southern to the northern. Whereas base on the recent reports these regions all present similar clinical symptoms on the JE patients.

Hence, in the following writing, a problem will be listed based on the backgrounds, with is more detailed analysis to confirm seasonal migratory birds are an important factor in the JE transmission as same as pigs, and also discuss the reason why other livestock or wildlife unable to play the same role as wild birds, what is the percentage the bird transmitted JE occupied in the JE infection origins and how can we enhance the precaution to JE through the analysis of bird migration. There are numbers of detailed problems wait to be solved throughout the project.

2. Case description

There are roughly 60 diseases are either directly or indirectly transmitted by birds. Not only seasonal migratory birds are able to transmit JEV, whereas most water areas beside residential portions are ideal place for both *Culex triaeniorhynchus* reproduction and wild bird living. It confirmed that the existence of birds in the local regions is vital to the epidemic, zoonotic diseases like JE cannot only be controlled from human aspects, but also accessible from reservoirs and medians. Combine with the current ecology of native birds in India that mainly distribute around water, they are the second possible object to be the JEV carrier while no piggeries instructing within residential areas. Additionally, the invention and popularize of JE vaccine for pigs decreased the seroprevalence of domestic pigs, which significantly descended the possibility JEV living on pigs [6]. Hence, it is equally important to focus on the reducing of the probability that JEV transmitted via wild birds, also for severe epidemics which mainly brought by JEV on wild those endemic regions with less distribution of piggeries [6,7]. Another seroprevalence test during 2005 to 2006 which carried out at Hokkaido (with least JE endemic area in Japan) showed 90% sera positive rate of specific JE antibodies in wild birds they caught, included wild ducks, *Anas acuta*, *Anas penelope* and *Anas poecilorhyncha* etc. through the analysis [7].

There are three approaches that wild birds associated with the JE transmission, which included birds with infectious JE symptoms bitten by *Culex tritaeniorhynchus* thus the latter carries the virus and transmit to human, birds with infectious JE symptoms directly transmit to human, and birds with infectious JE symptoms transferred to livestock around then the latter bitten by *Culex tritaeniorhynchus* and infect human.

Serology makes the JE infection detectable in human body hence it also can be used in livestock to test the presence of antigens. There are several sera positive tests of animals carried out in Malaysia 1953, which significantly showed that other than bovine (93%), equines (90%), only pigs came up with nearly 100% neutralizing antibodies for JEV. Wild birds were firstly confirmed that able to carry can spread JEV in 1958s, Japan, which indicated that wild birds are able to amplify JEV.[8] In addition, JE infected birds had similarities of the viremia with birds infected by flaviviruses, due to the property that flavivirus are transmitting via mosquitoes of *Culex* species, and mainly taking wild birds as reservoir host and amplifying reservoir [8]. After the JE was confirmed as insect-borne disease in 1970 in Malaysia, researchers also tested the sera positive rate of other animals, hence the seroprevalence rate of birds was noted as 16%, which is higher than the average in animals but much lower than pigs (46%) [8].

The main difference that makes birds stand out from bovines and equines is that JEV inside birds can cause high-concentration viremia inside the circulatory system, including viremic and non-viremic.

The main difference makes birds stand out from bovine and equines are that JEV inside birds is able to cause high concentration viremia inside the circulatory system. The investigation of clinical symptoms on birds associated with a process called Seroconversion. which is a more appropriate approach to justify the state of JE infection.[8] It can be defined as a turning point that specific antibodies presented inside the circulatory system. It was reported that seroconversion approximately happens after two weeks after the samples injected JEV genotype I or III, and the majority of the samples came up with viremia (97.3%). Another experiment carried out to investigate the impact of mosquitoes-transmitted JE on ducklings, which not only confirmed that most genotype of JEV are able to be transferred between adult birds and young birds without high fatality percentage, but also have 50%-100% possibility receiving and spreading JEV via *Culex tritaeniorhynchus* [7]. Either way, the previous studies offered a clue that either poultry and wild birds in any age are the most powerful reservoir hosts and amplifying reservoir compared with other livestock except pigs.

3. Correlation of waterfowl distribution and corresponding policies in states

In short, wild birds like water duck, common teals, pintail duck, cormorant, night heron, Indian shag, heron, egret, little cormorant are always available in Indian sanctuaries along the CAF during migratory season [9]. Those wetland sanctuaries supply appropriate environments for wild birds during the breeding season (June to July), such as heron, egret and little cormorant, whereas wild birds living in the freezing area like painted stork, Indian darters and Sarus cranes etc. will arrive at the southern India from Siberia before the winter, and stay in the sanctuaries for a period of time till the temperature in the high latitude area risen again [9]. Most bird sanctuaries open to the public for free, and the existence lasting impacting the wild bird distribution and the ecosystem surrounding residential quarters [9]. Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, West Bengal and Assam contributing to the majority of JE clinical cases, which is available in figure 4. Based on statistics, 72 bird sanctuaries established in India, which most sanctuaries are concentrate in JE endemic states such as Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Tamil Nadu and Uttar Pradesh etc.

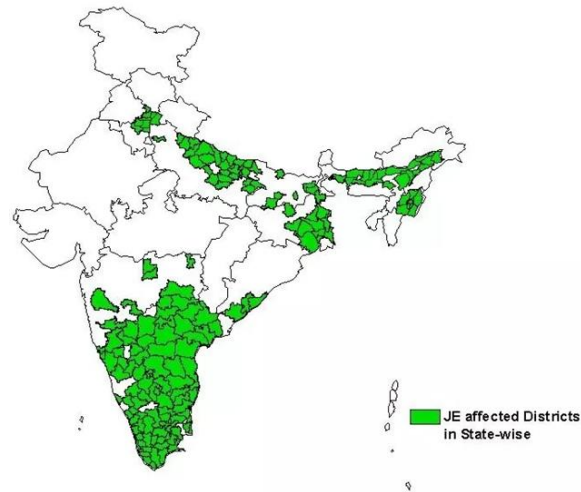


Figure 4. JE affected Districts in State-wise [2].

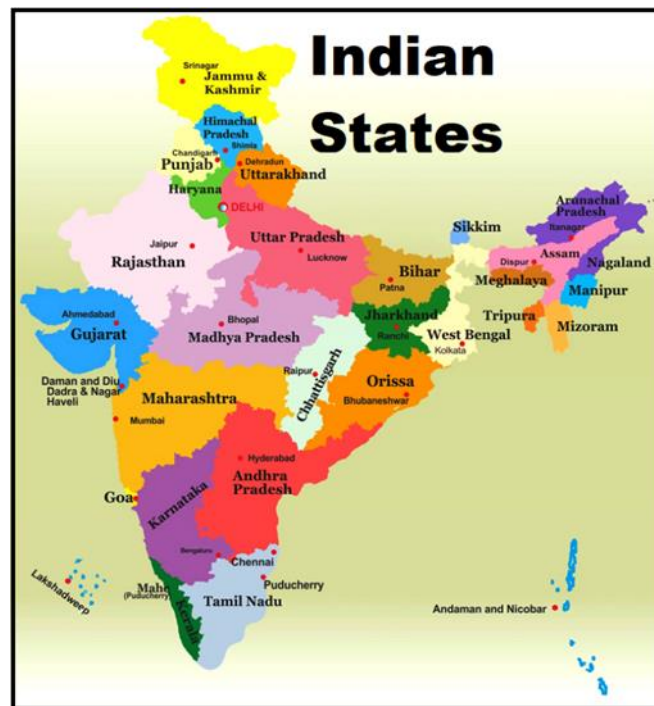


Figure 5. India States [9].

Hence wild bird is an important existence in the JE transmission process. Other than that, combine with the country we mainly focusing on, various factors strongly impacted the epidemic in local regions. Continuously drought and the changing climates caused more arid lands in major cities in the northern and western India. The severe condition strongly impacted the migration route of many migratory birds and the elimination of wild bird habitats, and also stimulated the establishment of policies about the instruction of reservoirs and artificial rivers. The corresponding actions and results included the disqualification of water resources around residential areas, mosquito growth in the stagnant water and changes of wild bird habitats etc., which ultimately leads to either the transmission of flavivirus (mainly spreading via mosquito) to non-endemic areas and amplification of the range of JE epidemic. For

example, North-South water transfer project since 1980s, this proposal was suggested for lessen droughts, though this also offered ideal water areas for mosquito growth.

In addition, the central Asian flyway (CAF) displayed the primary migratory routes of wild birds in central Asia, which involved the main land of southern India and the isolated islands chains in the southern British India Ocean (figure 6) [10]. Over 279 populations of 182 migratory species are participating in this route, also including approximately 16%(29) globally threatened and near-threatened species.[10] The destination for the most major winter waterbirds is the southernmost of the central Asia. and there are sanctuaries established along the route such as the Keoladeo National park, the outskirts of Mumbai, the Kutch Great Indian Bustard Sanctuary, Gujarat and Pathiramanal etc [10].

During the annual migration, the bird sanctuaries offered seasonal migratory birds a suitable condition to live, though for most of the wild birds they are temporary places for rest. For birds from the northernmost of Russia, they will crossover the whole Siberian plain then stay in the northern India, mainly in Uttar Pradesh. Apart from that, birds inhabit in China Tibet also have to cross the China-India border to arrive to the easternmost India, migrating to states like Assam, Arunachal Pradesh and Meghalaya etc. What information these factors referred to, is that the migrating routes of wild birds are playing an important role in the JE endemic areas. In short, this explained how can the JEV been spread to areas with less bird sanctuary distribution——foreign birds brought the virus whether they got infected in India or countries along the CNF to destinations they arriving at. For instance, this can be the reason why Assam contributing such a high JE case (~36%) in India [11].

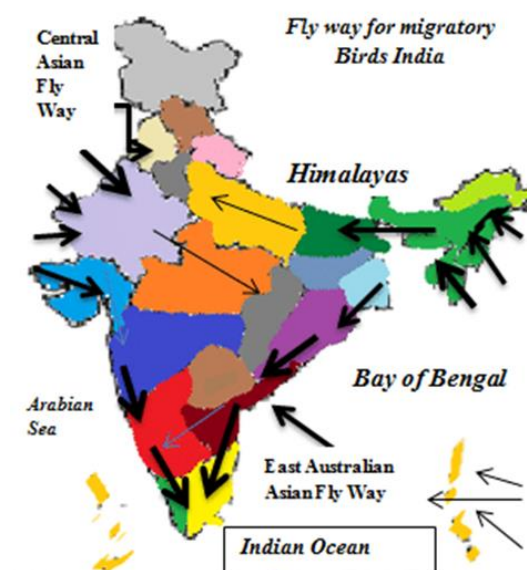


Figure 6. The central Asian flyway of wild birds in the main land of southern India and the isolated islands chains in the southern British India Ocean [10].

4. Current epidemic encephalitis B vaccine introduction

Most common types of JE vaccine for human worldwide are inactivated mouse brain-derived vaccines, inactivated Vero cell-derived vaccines, live attenuated vaccines, and live recombinant (chimeric) vaccines [4, 12].

Inactivated vaccine bases on the culture of the virus or pathogen, then inactivate them under specific temperature or chemical environment. The highlight of the manufacturing procedure is that fully identical virus or pathogen be produced but with no pathogenicity and virulence, thus it is able to trigger the humoral immunity and enables the antibodies created clean the poison in humor without cause severe immune response. The drawback emerged within the property of this type of vaccine, that it involves a large quantity of injection, shorter immune period and weaker resistance. To modify the current vaccine, live attenuated JE vaccine was invented for both stronger immunity and longer, lasting protection, that

it was suggested for children under 15-year-old. Since the JE vaccine injection policy established in 2014, children between 0-15 in 86% endemic area got least one dose, which assisted the inhibiting of potential JE epidemics.

Japanese Encephalitis Virus Vaccine Inactivated (JE-VAX), a typical JE precaution vaccine which was widely using in India before JE SA-14-14-2 vaccine was imported from China [10]. As a new live attenuated JE vaccine, it both presents stronger security and higher efficiency compared with the former. While JE SA-14-14-2 vaccine inducing into more states in India, the manufacture of JE-VAX was gradually shutoff [10]. The basic mechanism of JE SA-14-14-2 vaccine is called chimeric vaccine virus which is to use the yellow fever(YF) 17D as the base which coded with the gene sequence of the JE SA 14-14-2 strain to construct live attenuated vaccine, thus it can enhance the body immunize without cause damage to the blood brain barrier and leads to the destruction of neurons [12].

Thus, a problem emerged within the discovery, based on the tendency of the continuing epidemic in India, the JE SA-14-14-2 vaccine for human is suggested to get injection 1 or 2 months before the infection peak, or 1 or half month before visitors travelled to the epidemic areas. Two or more doses of JE SA-14-14-2 vaccine is involved in the state JE vaccination policy for children between 1-15, same as adults in the endemic areas. This ensured the resistance inside human body be enhanced. During the JE vaccination campaign in 2006, the free vaccination accesses and temporary vaccination clinics are available in particular states such as Assam, Uttar Pradesh (UP) and Tamil Nadu etc. For other states, JE vaccination for children is cost-free which already involved in the National Healthy Programme. However, for most adults the JE vaccine is both expensive and non-accessible. What reports showed is that approximately above 20% clinical cases are adults and the gender proportion are half and half. Due to the various factors, though the vaccination rate is high, the vaccine coverage control stuck at 39% and the vaccine efficiency of adults was descending since noted 90% at 2012. Hence it will be helpful if other approaches can be found to further restrict the JE epidemic.

5. Suggestions

To conclude with, it's either important for Indian citizens and the central Asia ecosystems to control the JE to avoid severe epidemics. Several advice are suggested to remit the infection and descend the possibility JE transmitting in a wider range, including screening the water resource distribution via satellite map to check if any idle lands beside residential areas have dirty water redundancy, and already have or have the potential to attract wild birds. This required to invite local staff to mark the areas where wild birds are most frequently available, thus the drainage system can be set up to avoid excess mosquito growth. For those outdoor water ponds that did not pass the water quality monitoring, the water area can be drained under the permission from residents then it can be used for other capital constructions. Clear procedures of submitting proposals to governmental administrations have to be constructed, and regular meetings need to be set up for discussing the content of the proposal.

Other than that, it will also help if the price of sanctuaries can be increased, for both decreasing the visitors and raise money for environment improvement and protection device establishment. Thus, the tourists have less possibility to get infection from the intimate contact with birds via biting by insects. Construct networks to monitor activities and traces of wild birds online, for response to the JE epidemic quicker and suspend the transmission between species. During the breeding period, frequent physical examination of both adult birds and birdling need to be planned as the regular conservation activity, for treating diseases and JE screening, which also assured a higher survival rate in birdling. From the aspect of feasibility, this either can be done by staffs from clinical institutions or professional researchers.

It will be a long-term program to make the suggestions become practical throughout the whole country. These proposals are considered to be the one has the highest feasibility. Other ideas such as the invention of JE vaccines for birds, the popularization of health management of poultry and so on, are not seems to be as possible as the former. One is because that improving the management of wild birds cannot generally making huge impacts on JE transmission, and it was came up with the idea to make it function in precaution aspects as far as it could. A few clinical spots will be set up in JE endemic areas under the approval of the proposals, for initiating long-term observation and data analysis. If the data

reflects that the annual JE cases, advanced cases and fatal cases is lower than the number from the deduction of the tendency, both the level of the impact and the feasibility of the proposal will be directly proved. Hence the scope and staff size can be expanded as the following step. Either way, the data and analysis which summarized in the text can offer a new approach for avoiding and inhibiting JE transmission, enhance people's understanding to JE and eliminate potential risks for individuals as much as possible.

6. Conclusions

In order to solve the problem of the year-round trend of JE, the text provides a clear indication of the history of the JE endemic in India by briefly describing and organizing the elemental backgrounds of JE and transmission sources. In addition to recent research, wild birds have been identified as a critical component of JE transmission, nearly as important as pigs. Despite this, they have long been disregarded while medical organizations continue to focus on the negative aspects of pig farming. This case study aimed to raise awareness of seasonal migratory birds, which are responsible for a significant number of infection cases in India and may increase bird-related JE infection in the future due to altered migration patterns brought on by global warming. It also discussed and compared the viability of recently established solutions in India with the above-mentioned personal suggestions. Errors and mistakes cannot be avoided as the actions have moved from the theoretical to the practical level. There is still room for improvement regarding the essay's errors and incompleteness in certain sections, and overall, the majority of these findings are tied to the advancement of technology in the future, making the outcome inexplicable.

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A discussion on the relationship between the five organs and proteinuria

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Abstract. Diabetic nephropathy is the most common kidney disease in recent years. In the early and middle stages of diabetic nephropathy, proteinuria is the main symptom, and it is speculated that proteinuria is caused by kidney essence deficiency. Proteinuria is widely recognized as an important indicator in the occurrence and development of various kidney diseases, and long-term proteinuria can cause serious harm to the body. Exploring the relationship between the five organs and proteinuria can help explore the mechanism of early proteinuria formation, propose effective predictive indicators, and facilitate timely clinical interventions to delay the occurrence and development of diabetic nephropathy. The etiology and pathogenesis of diabetic nephropathy are complex, and single treatment methods often fail to achieve ideal therapeutic effects. Traditional Chinese medicine emphasizes a holistic approach and individualized treatment, which can be beneficial in combination with modern Western medicine treatments. Research on the correlation between the five organs and proteinuria can further promote the integration of traditional Chinese and Western medicine in research and application, fully leveraging their advantages, and enhancing the efficacy of diabetic nephropathy treatment. The purpose of this article is to further strengthen the understanding of the relationship between the five organs and proteinuria, and provide theoretical basis and reference for the prevention and treatment of related conditions.

Keywords: Five organs; Proteinuria; Relationship; Academic discussion

1. Introduction

Proteinuria is a common clinical manifestation of kidney disease. When the qualitative test for protein in urine (such as acetic acid heating method, sulfosalicylic acid method, or protein qualitative test paper method) shows a positive result, it is referred to as proteinuria. Normal adult urine contains trace amounts of protein, and the normal range of urinary protein levels in healthy individuals is <150 mg/24 h urine [1]. Persistent proteinuria can cause damage to the kidneys, leading to glomerular sclerosis due to the kidneys being in a state of high perfusion, high osmotic pressure, and high pressure. Excessive reabsorption of protein by renal tubules leads to protein deposition in the epithelial cells of the renal tubules, causing tubular damage [2, 3]. Therefore, urinary protein can cause the continuous progression of kidney diseases and is an independent risk factor for the deterioration of renal function [4]. Proteinuria, as the most important manifestation of kidney disease, is closely related to its occurrence and development. However, it can also be a transient normal phenomenon in children. Intense physical activity, fever, and dehydration can also cause an increase in urinary protein levels. Recently, there have

been reports that the research focus has shifted from “proteinuria” to “urinary albumin.” Epidemiological studies in adults have shown a close relationship between increased urinary albumin and the occurrence of kidney and cardiovascular diseases [5].

Since the 1970s, Japan, South Korea, and China have conducted regular urine screening in schools, and it has been found that a considerable proportion of asymptomatic children have proteinuria. According to reports, screening conducted in Taiwan, China, among 3 million primary and secondary school students since 1990, showed that 5.81 individuals per ten thousand people had severe proteinuria [6]. This indicates that the incidence of proteinuria is increasing year by year. In traditional Chinese medicine theory, the kidneys are considered the main organ responsible for storing Yin essence, and proteinuria is considered a manifestation of Yin essence deficiency. By studying the relationship between the five organs and proteinuria, we can further clarify the etiology and pathogenesis of kidney-related proteinuria in traditional Chinese medicine and develop corresponding treatment systems. By observing the fluid flow between the kidneys and the bladder in the body, we can observe that they are very similar in color and odor, which suggests a close relationship between them. Moreover, the connection between the kidneys and the bladder can also be derived from the theories of traditional Chinese medicine’s zang-fu (viscera) theory. Ancient scholars recognized the mutual influence between the kidneys and the bladder, not only because of their proximity in terms of location but also because they are directly connected. Although the kidneys are located in front of the intestines anatomically, closer to the small and large intestines, they are not matched with the intestines. In addition, traditional Chinese medicine’s theory of zang-xiang (viscera and bowels) states that the kidneys govern water-fluid metabolism, control urine and feces, and are responsible for reproduction, which is closely related to the observed connection between the kidneys, bladder, and external genitalia by ancient scholars. This article aims to provide important theoretical and practical significance by deepening the understanding of the relationship between the five organs and proteinuria and their impact on diseases.

2. Understanding of Proteinuria in Traditional Chinese Medicine

In traditional Chinese medicine, there has never been a specific disease called “urine protein.” It is only mentioned in classical medical literature as “essence,” “fluid,” “liquid,” and “ointment.” Traditional Chinese medicine believes that essence, fluid, liquid, and ointment are general terms for certain “subtle substances” in the human body, apart from Qi and blood. When these “subtle substances” are lost and leaked, they can form proteinuria. The mechanism of proteinuria recognized in the current Chinese medicine field includes deficiencies in kidney essence storage, downward flow of essence, spleen deficiency in essence absorption, downward sinking of clear Qi, upward floating of turbid Qi, and lungs not governing Qi. However, the etiology and pathogenesis of spleen, lung, and kidney deficiencies vary from person to person and should be treated accordingly. Among the weakened organs, the spleen and kidneys are the most important. According to Shi Zhensheng [7], the presence of protein in urine indicates spleen and kidney deficiencies. Spleen deficiency results in improper transportation and failure to distinguish between clear and turbid, while kidney deficiency leads to the loss of Qi transformation authority and failure in essence storage, resulting in the leakage of subtle substances.

3. Definition and Functions of the Five Organs

“The Yellow Emperor’s Inner Canon: Su Wen - Differentiation of the Five Organs” also points out the relationship between the five organs, which are responsible for intake, and the six fu organs, which are responsible for excretion and transformation of substances. The coordination between the “ascending” function of the kidneys and the “descending” function of the six fu organs is necessary to achieve a balance in the metabolism of energy and substances. In the early and middle stages of diabetic nephropathy, proteinuria is one of the main clinical manifestations. Some scholars believe that the leakage of proteinuria is a manifestation of yin essence deficiency, and the storage, consolidation, and transformation of yin essence are mainly controlled by the kidneys. Therefore, insufficient kidney Qi and deficiency of kidney essence are one of the causes [8].

The five organs are important concepts in traditional Chinese medicine theory, referring to the heart, liver, spleen, lungs, and kidneys. They are considered essential components of the human body, playing irreplaceable physiological functions and regulatory roles. Each organ has its unique definition and function.

The heart is the ruler organ, responsible for the circulation of blood in the body and acts as a pump. Additionally, the heart is believed to be the center of emotions and consciousness. In traditional Chinese medicine theory, the heart is closely connected to mental activities, and the state of one's emotions directly affects heart health.

The liver is the general organ, considered as the main organ for storing and regulating blood. It not only maintains the balance of blood but also produces bile to assist in digestion and absorption. Furthermore, the liver is closely related to emotional regulation, and excessive excitement or depression can have adverse effects on the liver.

The spleen is the official of the granary, mainly responsible for digestion, absorption, and transportation of nutrients. It also plays a role in enhancing the body's immune system and resisting pathogenic factors.

The lungs are the prime minister organ, responsible for respiration and the transportation of Qi. Additionally, the lungs have the function of moisturizing the skin and regulating sweating, closely related to body temperature regulation.

The kidneys are the strong organ, considered as the storage site of essence and the regulator of water metabolism. They govern the growth, development, and reproductive ability of the body. Moreover, the kidneys are related to bone marrow and brain marrow, and they also have a certain influence on intelligence and memory.

The five organs hold a significant position in the human body, and each organ has its specific definition and function. They work together to maintain a balanced state of health. Understanding the definition and functions of the five organs is of certain significance for us to explore the relationship between the five organs and proteinuria.

4. Diagnosis and Clinical Manifestations of Proteinuria

The normal glomerular filtration membrane allows proteins with a molecular weight of 20,000-40,000 Daltons to pass through, while more than 90% of the proteins filtered by the glomerulus in the primary urine are reabsorbed by the proximal tubules. In a 24-hour urine collection, the total protein excretion in normal individuals should be below 150mg. In adolescents, the total protein excretion may be slightly higher but should not exceed 300mg/day, making it undetectable through conventional methods. Approximately 50-70% of urinary proteins are derived from plasma proteins, while the remaining 30-50% are proteins secreted by the urinary system, such as the loop of Henle and distal tubules, which secrete small amounts of mucoprotein and Tamm-Horsfall protein. These components make up trace amounts of protein. When the total protein in urine exceeds the aforementioned thresholds, it is referred to as proteinuria, which is a common clinical manifestation of kidney diseases [9].

The diagnosis and clinical manifestations of proteinuria are as follows:

(1) Urinary turbidity: Proteinuria refers to an elevated protein content in the urine. Normally, urine should be clear and transparent, but with increased protein levels, the urine becomes turbid.

(2) Increased sediment in urine: Proteinuria can also lead to an increase in sediment in the urine, which is typically observed as white, yellow, or red precipitates. These sediment particles contain a higher amount of protein.

(3) Edema: Proteinuria can cause fluid retention and impair kidney function, resulting in the accumulation of excess fluid in the body. Patients often experience swelling in the face, hands, feet, and may also exhibit pleural or abdominal effusion. In severe cases, it can lead to increased cardiac burden.

(4) Increased foamy urine: The presence of increased protein in the urine can make it more viscous, resulting in an increase in urinary foam that persists over time. The foam becomes denser when the urine is agitated.

(5) Other symptoms: Proteinuria may be accompanied by non-specific symptoms such as fatigue, loss of appetite, and weight loss. These symptoms are generally associated with metabolic disturbances caused by renal dysfunction.

In summary, proteinuria refers to an elevated protein content in the urine compared to normal values. Its clinical manifestations include urinary turbidity, increased sediment in urine, edema, and increased foamy urine. Proteinuria is often an early indicator of related diseases, and early detection and treatment are crucial in preventing disease progression.

5. The Connection between the Five Organs and Proteinuria

When studying the pathogenesis of proteinuria, we cannot overlook the close relationship between the five organs and proteinuria. The five organs are important organ systems in the human body, including the heart, lungs, liver, kidneys, and spleen. Each organ has its unique functions and roles, collectively maintaining the normal metabolism and function of the human body.

5.1. Relationship with the Kidneys

The kidneys play a crucial role among the five organs. The renal glomerulus is the basic structural unit of the kidneys and the primary site of proteinuria development. The glomerular filtration barrier consists of three layers: endothelial cells of the capillaries, basement membrane, and podocytes, which serve as a filtration mechanism. Under normal circumstances, only small molecular weight solutes and trace amounts of proteins can pass through this barrier, while large molecular weight proteins are prevented. When the glomerular filtration barrier is damaged, such as endothelial cell injury, thickening of the basement membrane, or podocyte detachment, it leads to an increased excretion of protein in the urine, resulting in the occurrence of proteinuria [9].

Furthermore, the renal tubules are important excretory pathways in the kidneys, participating in the reabsorption process of proteins. Under normal conditions, most of the proteins filtered into the renal tubules undergo reabsorption by the renal tubular epithelial cells, reducing the loss of proteins in the urine. Proteinuria is mainly caused by abnormalities in the structure and function of the glomerular filtration barrier and impairment of tubular reabsorption function, leading to increased excretion of protein in the urine [10].

In addition, the kidneys are also involved in the regulation of blood pressure, which is related to the occurrence of proteinuria. The kidneys regulate the constriction and dilation of the renal arteries, adjusting the glomerular filtration rate and perfusion pressure, thereby affecting the excretion of proteins. When kidney function is impaired, such as inadequate constriction of renal arteries or high perfusion pressure, it can lead to damage to the glomerular filtration barrier and an increase in protein loss in the urine, resulting in proteinuria [11].

In patients with chronic kidney disease (CKD) such as diabetic nephropathy, IgA nephropathy, focal segmental glomerulosclerosis, and hypertensive kidney injury, proteinuria is caused by immune and/or non-immune factors that lead to damage to the glomerular filtration barrier or renal tubules, resulting in the production of proteinuria.

Traditional Chinese medicine believes that dysfunction of the kidneys' solid storage function leads to the leakage of essence substances, and proteinuria is considered one of the essence substances. The relationship between solid storage function and proteinuria is explained from the physiological basis, diagnosis, and treatment of solid storage function dysfunction [12]. Therefore, all five organs have the nature of "master of storage" and should not be singularly attributed to the kidneys. By considering the connection between the kidneys and the essence stored by the five organs, it can be understood that the occurrence of essence leakage in other organs is also related to the kidneys.

5.2. Relationship with the Heart

The heart and the cardiovascular system are also crucial for maintaining normal kidney function. The heart pumps oxygenated blood to various organs in the body, including the kidneys. Nutrients and oxygen in the blood enter the kidney tissues through blood vessels, providing the necessary energy and

substances for renal units. Therefore, abnormal heart function or decreased blood flow may lead to inadequate blood supply to the kidneys, thereby affecting renal filtration function and causing proteinuria.

The presence of proteinuria indicates widespread dysfunction of the endothelium or systemic vascular disease. Dysfunction of the endothelium may promote increased leakage of lipoprotein particles into the arterial wall, ultimately leading to a significantly increased risk of atherosclerosis. It is considered an independent risk factor for cardiovascular disease and cardiovascular death in patients with type I and type II diabetes [13]. Whether proteinuria is a risk factor for cerebrovascular disease or simply a marker of cardiovascular-related diseases, it helps identify high-risk patients.

Heart diseases can cause changes in the permeability of the glomerular filtration membrane, leading to the occurrence of proteinuria by increasing the pressure within the kidneys. Heart failure is one of the most common heart diseases that can cause this condition. Chronic heart failure can lead to increased secretion of angiotensin in the body. However, angiotensin can bind to angiotensin receptors in the renal glomerular arteries, causing constriction and resulting in abnormal glomerular filtration, leading to proteinuria [14].

In addition, certain heart surgeries and interventions can also cause proteinuria. For example, coronary artery bypass grafting (CABG) is a common cardiac surgery used to treat coronary artery disease. Studies have found that after CABG surgery, patients have a higher incidence of proteinuria, which lasts for a longer duration. This may be due to factors such as increased aortic pressure during surgery and renal glomerular filtration membrane permeability changes caused by myocardial ischemia-reperfusion injury after surgery [15].

5.3. Relationship with the Liver

As an important metabolic organ in the body, liver disease can have certain effects on renal function, mainly manifested as proteinuria. The occurrence of proteinuria in liver diseases is generally associated with the following factors.

The liver plays a crucial role in the synthesis of plasma proteins, including albumin and globulin. Albumin is the most abundant protein in plasma and is primarily synthesized by the liver. All liver cells have the ability to synthesize albumin, but usually only one-third of them are active in this role, while the remaining two-thirds are in a resting state. In hypoalbuminemia, the synthesis rate of liver albumin increases, which is a key factor in the occurrence of compensatory reactions in the body. Studies have shown that liver albumin synthesis is mildly elevated in nephrotic syndrome, but the degree of elevation is often insufficient to compensate for urinary losses [16-17], suggesting that the compensatory capacity of liver albumin synthesis in kidney disease is strong but inhibited.

Liver disease can also cause kidney damage, leading to proteinuria. The liver is one of the main detoxification organs in the body, and when liver function is impaired, toxins can accumulate in the body. Some of these toxins are excreted through the kidneys. The accumulation of toxins can damage the glomerular filtration membrane, allowing protein molecules to filter into the urine, resulting in proteinuria.

Liver disease may also cause abnormal renal tubular function, leading to proteinuria. Renal tubules are responsible for the reabsorption of proteins in urine. In the case of liver disease, renal tubules may be damaged, reducing their reabsorption function, increasing the excretion of proteins in urine, and ultimately leading to proteinuria.

Liver disease may be related to inflammatory reactions, which can trigger renal damage and proteinuria to a certain extent. Research has found that liver inflammation is one of the important mechanisms by which liver disease causes proteinuria, damaging the glomerular filtration membrane and renal tubules.

5.4. Relationship with Other Organs

The association between proteinuria and diseases of other organs besides the kidneys, heart, and liver is worth further investigation. These organs include the spleen, lungs, stomach, pancreas, and intestines.

These organs play important roles in maintaining normal metabolic processes in the human body, and their relationship with proteinuria in diseases is also worth our attention.

Firstly, the connection between spleen disease and proteinuria has been confirmed in clinical practice. The spleen is an important immune organ in the body, responsible for the production of immune cells and the maintenance of immune function. When the spleen is diseased, it may lead to dysfunction of the spleen, which in turn affects the metabolism and clearance of proteins. Therefore, there is a certain connection between spleen disease and proteinuria.

Secondly, lung diseases are also associated with proteinuria. The lungs are vital respiratory organs in the human body, responsible not only for respiration but also for the elimination of waste gases and moisture. When the lungs are damaged, it may lead to abnormal protein metabolism in the body, resulting in proteinuria. The relationship between lung disease and proteinuria has received widespread attention in recent years, and important findings have been made in related research.

In addition, diseases of the digestive system organs such as the stomach, pancreas, and intestines are closely related to proteinuria. The stomach is the starting organ of the digestive process, while the pancreas is responsible for the secretion of digestive enzymes and the synthesis of insulin. When these organs are affected by disease, it may cause metabolic disorders, leading to the occurrence of proteinuria. Although there is still relatively limited research on the relationship between digestive system organs and proteinuria, some research results have indicated their association.

In conclusion, besides kidney, heart, and liver diseases, there is also a certain relationship between diseases of other organs and proteinuria. The connections between spleen diseases, lung diseases, and diseases of the digestive system organs, and proteinuria have been preliminarily confirmed in clinical practice, providing important insights for further exploration of the relationship between these organs and proteinuria. However, current research on these associations is still relatively limited, and further in-depth experimental studies and clinical observations are needed to further clarify the mechanisms and clinical significance of these relationships.

6. Treatment and Prevention of Proteinuria

6.1. Medication Treatment

In Western medicine, the control of blood pressure, blood sugar, and blood lipids is mainly achieved through dietary regulation. Additionally, antiplatelet or anticoagulant medications may be used, as well as hormones and immunosuppressants. Clinical trials have shown that reducing proteinuria can protect the kidneys, and it is believed that treating urinary protein can maximize kidney protection [18]. Glucocorticoids and immunosuppressants are used for etiological treatment. The preferred antihypertensive drugs are angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs).

ACEIs or ARBs, as angiotensin II receptor antagonists, have the functions of reducing glomerular pressure and decreasing urinary protein leakage. They can also inhibit abnormal proliferation of mesangial cells in the glomerulus, block the synthesis of extracellular matrix, delay the progression of glomerulosclerosis, downregulate the expression of transforming growth factors, and reduce the expression and deposition of extracellular matrix proteins such as glycogen and collagen [19]. They may also lower the levels of plasma inflammatory factors such as high-sensitivity C-reactive protein (hs-CRP), interleukin-2 (IL-2), and tumor necrosis factor- α (TNF- α), improve glomerular filtration rate, and reduce urinary protein excretion [20].

In theory, the combination of ACEIs and ARBs should achieve a synergistic effect. Previous clinical studies have also emphasized the use of combined therapy with ACEIs and ARBs, and it has been found that combined treatment has a stronger protein-lowering effect than high-dose monotherapy. Mongensen et al. [21] randomly divided 199 type 2 diabetic nephropathy (DN) patients into candesartan group, ramipril group, and combination therapy group. The results showed that the urinary albumin/creatinine ratio decreased by 24%, 39%, and 50% in the three groups, respectively. In a prospective randomized treatment of 24 non-DN patients, it was found that the urinary protein decreased by 56% in the

combination therapy group, 45.9% in the benazepril group, and 41.5% in the valsartan group; the urinary protein/creatinine ratio showed a similar trend. Currently, it is recommended to add ARBs or ACEIs to patients who do not respond to the maximum dose of ACEI or ARB in terms of protein reduction.

6.2. *Traditional Chinese Medicine Treatment for Proteinuria*

Although Western medicine has many treatment methods for proteinuria, their effectiveness is limited and they have significant side effects. Therefore, it is necessary to seek other safe and effective treatment methods. Traditional Chinese medicine (TCM) can play a “reducing toxicity and enhancing efficacy” role when used in combination with Western medicine, and it can also achieve better results when used alone. Most kidney disease patients have a long course of disease, during which their physical condition declines due to the long-term loss of “essential substances”. It is crucial not to neglect the presence of pathogenic factors in deficiency patterns. Therefore, the principle of treatment should be to tonify the body’s deficiency while dispelling pathogenic factors, i.e., not forgetting to expel pathogens while tonifying deficiencies and not neglecting deficiencies when expelling pathogens. Failure to follow this principle may lead to poor treatment outcomes or even worsen the condition. The kidney is the organ of water and fire, and the treatment should aim for a balance between water and fire. However, when the disease progresses to the late stage, the organs are vulnerable, and if medication is not taken with caution, it may result in overcompensation. Therefore, when selecting prescriptions and medications, it is essential to choose mild and balanced options and avoid aggressive approaches. In the treatment of proteinuria, it is important to use TCM for syndrome differentiation and treatment as much as possible. However, in necessary situations, it is still necessary to use Western medicine in combination, such as hormones and cytotoxic drugs. Timely use of Western medicine is still required for treatment. In TCM, proteinuria in kidney diseases is caused by multiple factors, involves multiple levels, and manifests in various attributes. Therefore, the key to treating proteinuria lies in distinguishing between deficiency and excess patterns. Since the treatment of this disease requires long-term medication, patients should be encouraged to adhere to the prescribed treatment, as relapses are common if medications are not taken consistently. In addition, as the condition gradually improves, the dosage can be reduced from once daily to once every other day, and the interval between medication can be gradually extended [22].

6.3. *Prevention of Proteinuria*

Prevention plays an important role in controlling the occurrence and development of proteinuria. Below, we will introduce several effective preventive measures from various aspects.

Firstly, a balanced diet is an important means of preventing proteinuria. Firstly, the intake of protein should be limited, as restricting the intake of protein in the diet can improve renal hemodynamics, reduce toxin accumulation, regulate ammonia metabolism by controlling the expression of multiple genes, reduce renal ammonia excretion, and prevent negative nitrogen balance [23]. Increasing the proportion of amino acids in the body can promote protein synthesis, improve metabolic abnormalities, protect residual renal function [24, 25], and help maintain renal autoregulation to counteract renal glomerular vasoconstriction and injury caused by hypertension [26]. Therefore, in daily diet, it is advisable to control the intake of high-protein foods such as meat and fish, and increase the intake of foods rich in fiber and antioxidants such as vegetables, fruits, and whole grains.

Secondly, salt intake should be moderately controlled in the diet. A high-salt diet can cause excessive sodium ion concentration in the body, leading to sodium and water retention, known as “sodium-water retention” in medicine [27]. High salt intake can increase renal blood flow and glomerular filtration rate, increase renal workload, and cause pathological changes in renal blood vessels [28], thus affecting renal function. Therefore, in daily diet, it is recommended to minimize salt intake, choose fresh foods, and avoid high-salt foods such as processed and canned foods.

Medication also plays a role in preventing proteinuria. Under the guidance of a doctor, appropriate medications can be used to control the occurrence and progression of proteinuria. Commonly used drug treatments include angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), which can inhibit the permeability of the glomerular filtration membrane, reduce protein

leakage, and thereby prevent and control proteinuria. Esnault et al. [28] found that diuretics combined with half-dose ACEI/ARB had better therapeutic effects in reducing urinary protein compared to the maximum antihypertensive dose of ACEI/ARB alone. In addition, drug treatments such as blood pressure regulation and glycemic control can also help prevent proteinuria.

Thirdly, developing good lifestyle habits is also very important in preventing proteinuria. Firstly, it is important to maintain good hydration habits and ensure an adequate intake of water every day, which helps dilute and eliminate metabolic waste products in the body and reduce the burden on the kidneys. Secondly, engaging in regular physical exercise to improve physical fitness and enhance immune function can help prevent proteinuria. At the same time, avoiding bad habits such as staying up late and excessive fatigue, maintaining sufficient sleep, is beneficial for body recovery and health maintenance.

In daily life, we should pay attention to the implementation of the above preventive measures. By adopting a balanced diet, medication treatment, and good lifestyle habits, we can help prevent the occurrence and development of proteinuria and maintain kidney health. Of course, specific treatment and prevention for proteinuria should be personalized according to the advice of a doctor. Only by considering multiple factors comprehensively and taking corresponding measures can we better prevent proteinuria and protect kidney function.

7. Conclusion and Prospects

In previous studies, we have conducted in-depth discussions on the relationship between the five organs (heart, liver, spleen, lungs, and kidneys) and proteinuria. However, there are still many issues that require further research. In future studies, we will focus on the following aspects: (1) Explore the specific mechanisms underlying the relationship between proteinuria and the five organs. Although we have clarified the connection between proteinuria and the five organs, we still lack a profound understanding of the specific molecular mechanisms involved. We will start from the cellular and molecular levels and use advanced biotechnology methods to further elucidate the interaction mechanisms. (2) Investigate the impact of different types of proteinuria on the five organs. Current research has mainly focused on common types of proteinuria, such as structural proteinuria and non-structural proteinuria. However, there are other types of proteinuria that deserve further investigation, such as microalbuminuria and glomerular proteinuria. This article will comprehensively examine the relationship between these different types of proteinuria and the five organs. (3) Explore the prospects of applying the research findings to clinical practice. The connection between the five organs and proteinuria is common to various diseases, such as diabetic nephropathy and proteinuric glomerulonephritis. Future research should focus on applying our research findings to clinical practice, providing efficient diagnostic and therapeutic methods for patient health. (4) Expand the research perspective and consider the effects of other influencing factors. The relationship between the five organs and proteinuria is complex, involving not only intrinsic organ factors but also lifestyle, environmental factors, genetic factors, and many other external factors. In future research, we will take these influencing factors into consideration to achieve a comprehensive understanding of the relationship between proteinuria and the five organs.

In summary, through in-depth research on the mechanisms of association, the relationship between different types of proteinuria, the application in clinical practice, and the consideration of other influencing factors, we will gain a deeper understanding of the association between the five organs and proteinuria. This will provide more effective methods and strategies for disease prevention and treatment. Future research will further reveal the profound connection between the five organs and proteinuria and have a greater impact on human health.

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The study on the relationship between dementia and air pollution

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Abstract. Dementia is a multifaceted neurological condition distinguished by a reduction in cognitive abilities, impairment of memory function, and worsening in overall functional capacity. In recent times, there has been an increasing level of apprehension regarding the potential consequences of environmental elements, specifically air pollution, on the advancement and manifestation of dementia. The primary objective of this research article is to investigate the correlation between dementia and air pollution by a comprehensive analysis of epidemiological data. Additionally, this study will delve into probable processes that may underlie this association and then analyze the ramifications for public health. The results indicate that the presence of air pollution could potentially be a contributing factor to an elevated susceptibility to dementia and a deterioration in cognitive function among those afflicted. Gaining a comprehensive understanding of this correlation is of utmost importance in order to formulate efficacious preventive measures and policies aimed at mitigating the adverse effects of air pollution on dementia.

Keywords: dementia, air pollution, neurodegenerative disorder, cognitive decline, epidemiology, public health

1. Introduction

Dementia poses a substantial worldwide health burden, carrying notable societal, economic, and healthcare ramifications. The condition is distinguished by a reduction in cognitive functions, encompassing memory, cognition, rationality, and conduct. With the aging population and the rise of urbanization, there is a mounting apprehension regarding the influence of environmental variables on the risk and advancement of dementia. One of the environmental variables that has garnered significant attention is air pollution, mostly due to its extensive prevalence and potential detrimental impacts on human health. The primary objective of this study is to investigate the correlation between dementia and air pollution. The primary objective of this study is to conduct a comprehensive examination of the current epidemiological data that establishes a connection between exposure to air pollution and the development of dementia. Additionally, this research aims to investigate various processes that may explain this association, analyze the consequences for public health, and propose recommendations for preventive methods and policies.

2. Dementia: Definition, Types, and Prevalence

2.1. Overview of Dementia

Dementia is a clinical condition distinguished by a gradual deterioration of cognitive abilities, resulting in impaired performance of everyday activities. The etiology of this condition can be attributed to a range of underlying diseases, such as Alzheimer's disease, vascular dementia, Lewy body dementia, and frontotemporal dementia. The hallmark features of dementia include memory loss, impaired judgment, language difficulties, and changes in behavior and personality.

2.2. Common Types of Dementia

Alzheimer's disease is the prevailing form of dementia, including around 60-80% of reported instances. Vascular dementia, characterized by cerebral hypoperfusion, ranks as the second most frequent kind. Lewy body dementia and frontotemporal dementia are characterized by unique pathological characteristics and clinical presentations.

2.3. Global Prevalence and Burden

Dementia presents a substantial global health dilemma, since around 50 million individuals are presently afflicted with this disorder on a global scale. The anticipated increase in the prevalence of dementia is closely correlated with the aging population. The economic and social impact of dementia is considerable, imposing a huge burden on healthcare institutions, caregivers, and society at large.

3. Air Pollution: Types, Sources, and Composition

3.1. Types of Air Pollutants

Air pollution is composed of a multifaceted combination of particulate matter (PM), gaseous pollutants, and various other harmful elements. Particulate matter (PM) is classified according to its size, wherein PM_{2.5} (particles with a diameter of 2.5 micrometers or less) and PM₁₀ (particles with a diameter of 10 micrometers or less) are of significant interest due to their capacity to infiltrate the respiratory system at a profound level.

3.2. Major Sources of Air Pollution

Air pollution originates from both outdoor and indoor sources. Outdoor sources include industrial emissions, vehicle exhaust, power plants, and agricultural activities. Indoor sources include combustion of solid fuels for cooking and heating, tobacco smoke, and building materials.

3.3. Composition and Health Effects of Air Pollutants

Air pollutants encompass a diverse array of substances, including nitrogen dioxide (NO₂), sulfur dioxide (SO₂), carbon monoxide (CO), ozone (O₃), volatile organic compounds (VOCs), and heavy metals. These pollutants have been linked to a range of negative health impacts, such as respiratory and cardiovascular ailments, as well as possible neurotoxic effects.

4. Epidemiological Studies on Air Pollution and Dementia

Several longitudinal cohort studies have been undertaken to examine the correlation between air pollution and the development of dementia. These investigations involve the longitudinal tracking of a substantial cohort of individuals for an extended duration, with the aim of evaluating their level of exposure to atmospheric pollution and its potential correlation with the subsequent onset and progression of dementia. Numerous cohort studies have consistently documented a strong correlation between prolonged exposure to air pollution and an elevated susceptibility to dementia. An investigation conducted by Chen et al. scrutinized a population of more than six million inhabitants in Ontario, Canada, and revealed that persons residing within a proximity of 50 meters from main roadways had a considerably elevated susceptibility to acquiring dementia in comparison to those

residing at greater distances [1]. The study additionally exhibited a dose-response correlation, wherein a higher risk of dementia was identified in those living in closer proximity to major roadways characterized by elevated levels of air pollution originating from traffic. In a similar vein, a comprehensive cohort study was out in Sweden by Oudin et al. meticulously tracked the progress of more than 3,000 individuals over an average duration of 11 years. The research conducted revealed a positive correlation between elevated levels of chronic exposure to air pollution originating from traffic, namely nitrogen oxide (NO_x) and particulate matter (PM_{2.5}), and a heightened susceptibility to dementia, with a particular emphasis on Alzheimer's disease and vascular dementia [2].

Case-control studies have made significant contributions to the comprehension of the association between air pollution and dementia. These studies involve the comparison of individuals diagnosed with dementia (referred to as cases) to individuals who do not have dementia (referred to as controls), with the aim of evaluating their prior exposure to air pollution. Case-control studies offer supplementary evidence about the potential association between exposure to air pollution and the risk of developing dementia. An investigation conducted by Chang et al. in Taiwan utilized a case-control study design to examine the potential correlation between prolonged exposure to fine particulate matter (PM_{2.5}) and the likelihood of developing dementia. The research encompassed a sample size of more than 12,000 instances of dementia, along with corresponding control groups. The findings of this study revealed a noteworthy and positive correlation between exposure to PM_{2.5} and the likelihood of developing dementia. The results indicated that prolonged exposure to PM_{2.5} was associated with an elevated likelihood of acquiring dementia, particularly among persons with genetic predisposition [3].

Ecological investigations have been conducted to examine the correlation between levels of air pollution and the prevalence or incidence rates of dementia within a population. These studies offer significant insights into the potential influence of air pollution on the outcomes of dementia. Wu et al. conducted a noteworthy ecological study in the United States to investigate the correlation between air pollution at the county level and the prevalence of dementia. The present study conducted an analysis of data obtained from a sample of more than 6,000 counties, revealing a positive correlation between elevated levels of PM_{2.5} and nitrogen dioxide (NO₂) and an increased prevalence of dementia. The study posited that the reduction of air pollution levels on a regional scale may potentially have a mitigating effect on the burden of dementia [4].

The synthesis of findings from many epidemiological research through meta-analyses and systematic reviews offers a complete evaluation of the association between air pollution and dementia. The aforementioned analyses have provided more evidence to substantiate the correlation between exposure to air pollution and a heightened susceptibility to dementia. An investigation conducted by Peters et al. encompassed a systematic review and meta-analysis of 14 papers. The findings of this study indicated that prolonged exposure to air pollution, specifically PM_{2.5} and NO_x, exhibited a positive correlation with a heightened susceptibility to dementia. The findings of the analysis demonstrated a consistent and positive correlation across various study designs and populations, thereby enhancing the body of evidence supporting the adverse effects of air pollution on dementia [5].

The available epidemiological studies collectively indicate that prolonged exposure to air pollution, specifically PM_{2.5} and contaminants connected to transportation, is correlated with a heightened likelihood of developing dementia. The results of this study offer valuable insights into the possible influence of air pollution as a modifiable risk factor for dementia. These findings underscore the necessity of implementing preventative measures and public health interventions to reduce the adverse effects of air pollution on cognitive health [6-8].

5. Potential Mechanisms

In order to comprehend the correlation between air pollution and dementia, it is imperative to investigate the plausible biological pathways by which air pollution can manifest its deleterious impacts on the brain. Several mechanisms have been proposed based on experimental and observational studies, shedding light on the possible pathways linking air pollution to dementia.

Neuroinflammation has been identified as a significant factor involved in the correlation between air pollution and the development of dementia. The presence of air pollutants has the potential to induce an inflammatory reaction within the brain, which is distinguished by the activation of microglia and the subsequent release of pro-inflammatory cytokines and chemokines. Chronic neuroinflammation has the potential to induce neuronal damage, disrupt synaptic function, and ultimately play a role in the pathogenesis and advancement of dementia. Empirical investigations have provided evidence indicating that the introduction of air pollutants, namely fine particulate matter (PM_{2.5}) and pollutants associated with transportation, can elicit neuroinflammatory reactions in animal subjects. The observed reactions are linked to the build-up of neuroinflammatory indicators, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and cyclooxygenase-2 (COX-2). The initiation of inflammatory pathways has the potential to disturb regular neuronal function and play a role in the process of neurodegeneration [7, 9, 10].

Another important mechanism by which air pollution may contribute to dementia is oxidative stress. Air pollutants contain reactive oxygen species (ROS) and other toxic compounds that can induce oxidative damage in the brain. Oxidative stress arises from a state of disequilibrium between the generation of reactive oxygen species (ROS) and the body's capacity to counteract them through antioxidant defense mechanisms. This imbalance results in cellular harm and compromised neural functionality. Numerous investigations conducted on animals and humans have demonstrated that exposure to air pollution leads to an elevation in oxidative stress markers. Cellular structures and functions can be adversely affected by oxidative damage to lipids, proteins, and DNA. In addition, the presence of oxidative stress can trigger the initiation of inflammatory pathways and the production of neurotoxic compounds, so intensifying the progression of neurodegenerative mechanisms [7, 10].

Exposure to air pollution has been found to be linked with systemic inflammation, impairment of endothelial function, and an elevated risk of cardiovascular disease. The presence of vascular factors has been identified as a potential contributing element in the pathogenesis of cerebrovascular disease, a condition that has been associated with an elevated susceptibility to vascular dementia and cognitive deterioration. The presence of air pollutants, specifically particles resulting from combustion, has the potential to infiltrate the bloodstream and directly impact the vascular function within the brain. Numerous empirical investigations have substantiated the correlation between prolonged exposure to atmospheric pollution and an elevated susceptibility to stroke and cerebrovascular ailments. The disruption of cerebral blood flow, reduced oxygen supply, and compromised vascular integrity can contribute to neurodegenerative processes and the onset of dementia [6-8].

The blood-brain barrier (BBB) serves as a crucial protective mechanism that governs the selective transport of chemicals between the vascular system and the central nervous system. The function of this mechanism is of utmost importance in the regulation of brain homeostasis and the safeguarding of the brain from potentially detrimental agents. Airborne pollutants, including ultrafine particles, have the ability to directly traverse the blood-brain barrier (BBB) and get access to the brain. Research has demonstrated that the presence of air pollution has a detrimental effect on the integrity of the blood-brain barrier (BBB), resulting in heightened permeability and facilitating the infiltration of harmful substances into the brain. The aforementioned disturbance has the potential to induce harm to neurons, provoke inflammation, and impair neuronal function, hence playing a role in the progression of dementia [6-8].

The sensitivity of individuals to the detrimental impacts of air pollution on the brain might be influenced by hereditary factors at the individual level. The response of individuals to air pollution exposure can be influenced by genetic variations in genes associated with inflammation, oxidative stress, and detoxification pathways. The potential for gene-environment interactions, namely those involving genetic variations associated with inflammation or oxidative stress and exposure to air pollution, may contribute to an elevated susceptibility to dementia [7].

Through genetic studies, researchers have successfully identified distinct genetic variations that have the potential to alter the relationship between air pollution and cognitive outcomes. The results of

this study indicate that the impact of air pollution on brain function may be influenced by hereditary factors, leading to variations in individual sensitivity [7].

Gaining a comprehensive understanding of the fundamental mechanisms that establish a connection between air pollution and dementia is crucial in order to facilitate the development of precise interventions and preventive measures. Through the clarification of these mechanisms, researchers are able to ascertain prospective treatment targets and formulate solutions to alleviate the deleterious impacts of air pollution on cognitive well-being.

6. Implications for Public Health

First, to mitigate the impact of air pollution on dementia, policymakers need to implement effective strategies to reduce air pollution levels. This includes stricter emissions standards for industrial and transportation sectors, promoting clean energy sources, and fostering sustainable urban planning.

Second, it is imperative to acknowledge the significant impact that public education and awareness campaigns may have in disseminating vital information to the broader population regarding the possible hazards associated with air pollution and its detrimental effects on cognitive well-being.

Third, continuous efforts are needed to advance environmental regulations and standards to ensure the protection of public health. Implementing and enforcing stricter air quality standards can significantly reduce the population's exposure to harmful air pollutants.

Urban planning initiatives that prioritize green spaces, pedestrian-friendly infrastructure, and efficient public transportation systems can help reduce air pollution levels in urban areas. Research has demonstrated that the presence of green areas has a beneficial effect on cognitive function and mental well-being.

Fourth, addressing the complex relationship between air pollution and dementia requires interdisciplinary collaborations among researchers, healthcare professionals, policymakers, and community stakeholders. Such collaborations can facilitate the development of comprehensive strategies for prevention, intervention, and policy implementation.

7. Challenges and Future Directions

Epidemiological investigations that explore the association between air pollution and dementia encounter several methodological obstacles, encompassing the accurate measurement of exposure, the presence of confounding variables, and the possibility of reverse causation. By addressing these shortcomings, the evidence base can be strengthened and our knowledge of the association can be improved. Certain demographic groups may exhibit increased susceptibility to the detrimental impacts of air pollution on the development and progression of dementia. The identification of vulnerable populations, including older adults, persons with pre-existing cognitive impairments, and those with genetic predispositions, can facilitate the implementation of focused interventions and preventive measures.

Additional investigation is required to delve into the enduring consequences of exposure to air pollution on the chance of developing dementia, as well as to determine the correlations between the dosage of air pollution and its corresponding effects. The utilization of improved air pollution monitoring systems and other enhanced exposure assessment methodologies will contribute to a deeper comprehension of the cumulative impacts of air pollution. The exploration of the fundamental processes connecting air pollution and dementia necessitates the conduct of mechanistic investigations and the discernment of biomarkers. The examination of neuroinflammatory indicators, oxidative stress markers, and imaging techniques can yield valuable insights into the precise mechanisms by which air pollution impacts the brain. Subsequent investigations ought to prioritize the assessment of the efficacy of therapies and preventive measures targeted at mitigating the deleterious consequences of air pollution on the development and progression of dementia. This encompasses the examination of the advantages associated with indoor air filtration systems, adjustments made to one's lifestyle, and specific therapies designed for populations at a heightened risk.

8. Conclusion

The available literature indicates that there is a mounting body of data supporting the notion that the exposure to air pollution is correlated with an elevated likelihood of developing dementia and experiencing a deterioration in cognitive function. Gaining a comprehensive understanding of the correlation between air pollution and dementia is of paramount importance in order to facilitate the development of efficacious preventative measures, the implementation of environmental legislation, and the advancement of public health initiatives. Through the acknowledgment and examination of the various obstacles, as well as the allocation of resources towards additional investigation, scholars can strive to mitigate the detrimental impact of air pollution on dementia and enhance the well-being of those afflicted by this incapacitating ailment.

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Bacteria mediated cancer treatment (BMCT) and quorum sensing system in BMCT

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Abstract. Many treatments against cancer already exist. For example, radiation therapy, chemical therapy and immunotherapy, combined the physical surgery, these treatments do make a remarkable effect in inhibiting the growth of the tumor and thus prolonging the patient's life. However, most of the treatments mentioned above would simultaneously damage normal cells and cause trauma or pain to the patients. In recent years, researchers have turned their attention to the remarkable natural abilities of certain bacteria such as Salmonella and E. coli, in suppressing malignant cells and solid tumors. Bacteria-mediated cancer treatment (BMCT) has emerged as a promising approach that offers the potential for reduced patient trauma and enhanced precision in targeting cancerous tissues. Furthermore, the integration of quorum sensing systems, initially utilized in the fermentation process holds promise in augmenting the navigational capabilities of bacteria. This advance opens the door to the development of highly location-sensitive bio-agents capable of delivering substantial quantities of specific therapeutic proteins directly to tumor sites. This review aims to comprehensively examine existing BMCT methodologies and explore the quorum sensing pathways that have been identified. Additionally, it contemplates the feasibility of synergizing these two systems to offer a novel perspective on cancer treatment.

Keywords: Quorum sensing, Salmonella, Bacteria-mediated cancer treatment, colorectal cancer

1. Introduction

Up to these days, people have discovered numerous effective methods for treating cancer, including but not limited to chemotherapy, radiation therapy, and surgical removal. However, these treatments often inflict significant damage on fragile patients. Much of the unnecessary harm arises from the inability of traditional treatment to target specific areas. While these 'scorched earth' approaches do effectively eliminate cancerous lesions, they also diminish patients' living quality, in some degrees contradicting the fundamental purpose of healthcare - to alleviate suffering. Therefore, humans have tirelessly sought more localized treatment methods. In recent years, a treatment called Bacterial-Mediated Cancer Therapy (BMCT) has come into view. BMCT leverages the ability of certain bacteria to locate tumors, along with other clinically advantageous characteristics of these bacteria. Coupled with emerging gene-editing technologies, it offers a more precise approach to treat cancer. However, there currently exists a dearth of comprehensive reviews on the underlying systems of BMCT. Thus, this review aims to cover as many of the systems and bacterial strains on which BMCT relies as

possible. Additionally, it will enumerate existing experimental progress and propose potential mechanisms for anti-cancer action. It is our hope that this review will provide future researchers with insights to achieve greater strides in BMCT research.

1.1. Introduction of bacteria-mediated cancer treatment (BMCT)

In the late 19th century, Dr. William Coley and his research team pioneered the utilization of bacteria in the treatment of cancer. A patient diagnosed with advanced bowel cancer was referred to the emergency room where medical professionals conveyed the grim prognosis of an imminent demise within a year, as conventional treatment appeared futile in addressing the tumor. However, a remarkable turn of events transpired after the patient was infected by acute erysipelas. This medical anomaly piqued the interest of Dr. Coley who then embarked on reviews on more patients who experienced similar recoveries and noticed a correlation between bacterial infection and tumor regression.

Subsequently, he thus formulated a therapeutic approach involving the development of what would later become known as “Coley toxins” which were concocted by blending inactivated *Streptococcus pyogenes* and *Serratia marcescens*

bacteria, and he then administered this toxin through direct injection into the tumors to treat more than a thousand cancer patients. Even though the five-year survival rate remarkably increased under this treatment, it was apparent that a majority of the treated individuals endured ongoing health challenges and faced a limited life expectancy.

After the invention of chemotherapy and radio treatment temporarily overshadowed the pursuit of BMCT, the study of using bacteria toxins to treat cancer was suspended. Nevertheless, as scientific understanding of immunology advanced over the years, Dr. Richardson and his coworkers revisited Doctor Coley’s pioneering work. They compared the effectiveness of Coley’s toxin with contemporary cancer therapies based on published results. It turns out that the Coley toxin could behave as the pathogen-associated molecular patterns (PAMPs), recruiting immune cells such as the dendritic cells, neutrophils, and the natural killer cells to the tumor sites, consequently inciting an inflammation that played a pivotal role in cancer resolution. [1-3]

However, the initial application of the bacterial toxins carried a significant risk of inducing severe infections. Fortunately, advancements in genetic engineering technology enabled the achievement of modified strains, producing toxicity-reduced bacterial toxins. Notable bacterial species, including BCG, ST., *Clostridium*, *E.coli*, *Listeria*, *Monocytogens* and *Shigella* have been harnessed in the production of cancer vaccines, offering promising prospects for the future of cancer immunotherapy. [2, 4]

BMCT presents distinct advantages in treating cancer juxtaposed with other traditional cancer therapies. Chemotherapy, a widely used and efficacious approach to cancer treatment, entails the systemic administration of therapeutic agents and therefore treats tumors distributed throughout the patient’s body. However, chemotherapy is associated with deleterious side effects such as alopecia due to follicle cell damage, gastrointestinal complications, and myelosuppression, leading to a diminished count of immune cells and platelets. One important reason contributing to the multifarious side effects is the passive diffusion-dependent nature of therapeutic chemicals. The chemicals lack the ability locating to nidus, necessitating the administration of substantial quantities of these chemistry agents to ensure the treatment efficacy. In stark contrast, anaerobic bacteria thriving exclusively within the hypoxic microenvironment at the central hollow part inside the tumors, offer an inherently site-specific modality for cancer intervention. [5] This inherent localization within the central hollow region of the tumor affords the unique advantage of mitigating risks to surrounding healthy tissues, thereby significantly reducing the potential for collateral damage in BMCT-based cancer treatment strategies. [2, 4]

Additionally, it is noteworthy that traditional therapies, reliant on systemic circulation for drug delivery, may encounter limitations in addressing early-stage tumors situated at a considerable distance from vessels. In contrast, as bacteria are relatively metabolic independent, they could infiltrate

malignant tissues deeply from vascular proximity, thereby extending their therapeutic reach within the context of cancer treatment.[2]

Similar to the pioneering work of Dr. Coley, bacteria exert their immunomodulatory influence by eliciting localized inflammation within tumor regions. A fundamental issue contributing to unbridled tumor growth resides in the phenomenon known as the tumor immune escape effect. Within this context, malignant cells turn down their expression of the major histocompatibility complex (MHC) molecules on their membranes and concurrently exhibit reduced level expression of the costimulatory proteins responsible for the secondary activation signal crucial for converting naive T cells into cytotoxic T cells. Consequently, this deceptive alteration leads the immune system to erroneously perceive tumor cells as normal tissue cells assisting the immune surveillance evasion of tumor cells.

Besides, certain tumor cells release immunosuppression molecules attracting regulatory T cells orchestrating the expulsion of other immune cell populations aimed at targeting the tumor. However, several structural components and unmethylated proteins present on the bacterial cell wall could send strong signals to the immune system informing a severe intrusion within the affected area. Then, the toll-like receptors on immune cells recognize these bacterial proteins, further activating the innate and adaptive immune response, which manifests through an augmented production of several inflammatory cytokines such as IL-12 [6].

1.2. *Salmonella* in BMCT

Among all bacteria, *Salmonella* exhibits an outstanding feature as a promising candidate for advanced tumor treatment, primarily owing to its amenability to genetic manipulation. The well-characterized expression systems in *Salmonella* have enabled the development of some ideally engineered strains such as the *Salmonella* Typhimurium VNP20009, A1-R (eu-/arg-), SHJ2037 (relA-/spoT-) [7-8].

As previously outlined in the 1.1 paragraph, *Salmonella* shared several advantages inherent to BMCT such as locating to tumor and providing site-specific treatment, triggering localized inflammation, penetrating into the tumor tissue, and operating independently off the circulatory system.[1-3, 5, 6, 9] In addition to these common attributes, *Salmonella* possesses some unique merits. It can directly eliminate cancer cells through mechanisms that remain not fully elucidated. The AI-1 that *Salmonella* produced in its quorum sensing system, for example, has been observed to induce tumor cell death within a remarkably short timeframe of around thirty minutes. Furthermore, when *Salmonella* competes for the nutrients with tumor cells, it simultaneously produces toxins that instigate the apoptosis process in cancer cells by promoting the degradation of long-lived cytoplasmic proteins.[10]By turning down the expression of mTOR and the protein kinase B, *Salmonella* can suppress the expression of the HIF-1 α which results in the reduction of VEGF disrupting the tumor cells' capacity to recruit blood vessels [2, 7, 11-13].

Another notable feature of *Salmonella* is its effectiveness by oral administration, allowing it to carry much of the chemicals to the tumor when it is acting as a carrier [14]. Moreover, advancements in modifying *Salmonella*'s quorum sensing system offer the prospect of finely regulating the timeline for production, release, and clearance, enhancing its therapeutic potential [7, 12, 13].

Possessing these adorable advantages in treating cancer, *Salmonella* has therefore already been used either to kill the tumor cells directly using the toxins it produced, or to induce inflammation, or to deliver therapeutic chemicals as carriers to tumor sites serving as a replacement for chemotherapy requiring injecting large amounts of the chemicals.[11] These applications have demonstrated significant potential in treating several types of cancers such as melanoma, liver cancer, breast cancer, prostate cancer, and colorectal cancer [2, 7, 12].

Even more, *Salmonella* is currently being investigated in clinical trials in treating pancreatic cancer. As an anaerobic bacterium, *Salmonella* exhibits better efficiency in addressing solid cancers. Yet, most clinical trials using *Salmonella* as cancer treatment are still in their first or second phases. The utilization of *Salmonella* as a therapeutic agent requires the patients not to have resistance to any kind of antibiotics since it is important to maintain control over the *Salmonella* population to prevent uncontrolled over-proliferation and the potential development of septicemia. Also, researchers face the

challenge of striking a delicate balance in the amount of natural toxins that *Salmonella* can carry. Excessive reduction of toxins may compromise its effectiveness in treating cancer, while retaining too many toxins could pose a risk of severe infection [7, 12].

However, *Salmonella* has not been discovered with the ability to completely eliminate all tumors in patients on its own. The BMCT based on *Salmonella* needs to cooperate with other conventional cancer treatments, such as administering effective medications or surgically removing some sizable tumors [7, 12].

In a phase I clinical trial report posted in 2002 by Toso et al., VNP20009 was used to treat patients with metastatic melanoma or renal cell carcinoma [15]. The deletion of the *msbB* and *purI* genes is conducted in order to reduce the overall toxicity of VNP2009 rendering it safe for intravenous infusion. The deletion of the *msbB* gene served to prevent the combination of the myristyl group with the lipid A domain of the lipopolysaccharides (LPS), which could reduce the toxicity of LPS of VNP20009, whereas the removal of the *purI* necessitated the provision of exogenous adenine which could contribute to its capacity to locate as well as reduce the possibility inducing bacteremia [6, 16].

The clinical trial incorporated a total of 24 patients who were intravenously injected with different doses of the VNP20009 strain. Bacterial concentrations were closely monitored in the patients' blood, urine, feces, and tumor sites. Since the VNP20009 strain did not carry additional chemicals, this trial's approach focused on inducing localized inflammation in tumor sites and thus facilitating the introduction of tumor necrosis factor-alpha (TNF- α) to trigger tumor auto-apoptosis [7].

However, the outcome of this clinical trial proved to be less than satisfactory. Approximately one-sixth of the patients had experienced prolonged low-concentration bacteremia without definitive indication of bacterial residence in the tumor site. Besides, the shrinkage of the tumor was not obviously observed. Therefore, even though, in many animal experiments, the concentration of VNP20009 in tumor sites, compared to its concentration in blood or other tissues, could be 1000 times higher, the bacteria's colonization ability in the human body remained uncertain. The research team also explored the maximum tolerated dose (MTD) by assessing the dose limiting toxicity (DLT) response, in which they found the original toxicity of *Salmonella* constrained the MTD, turning the antibiotics treatment a required addressing process after the experimental trial ended. In cases where four patients displayed symptoms indicative of low-concentration bacteremia, an immediate antibiotic injection was administered to eliminate any potential bacterial threats.

In another clinical trial conducted in 2008, advanced tumor patients were treated with a further modified VNP20009 strain which, in addition to the deletion of the *msbB* and *purI* genes, integrated the *CD* gene, a gene coding for an enzyme absent in mammalian cells [6, 16-18]. This enzyme had the unique ability to convert 5-FC protein into 5-FU protein. The product, 5-FU protein, acting as a cytotoxic antimetabolic agent within tumor sites, was released by *Salmonella*, and could theoretically curtail the growth of tumor cells. This treatment using the modified VNP20009 strain was further named TAPET-CD. Instead of intravenous injection, the research group experimented with shallow direct injections around the tumor to avoid the diffusion of the bacteria thereby reducing the risks of getting bacteremia. During the clinical trial, three patients were treated with bacteria that did not reach MTD, alongside highly concentrated 5-FC molecules.

Although one of the patients showed a notably high concentration of 5-FU in the tumor site, this patient had experienced a grade 3 toxic event prompting their withdrawal from the experiment prematurely, and sadly this patient passed away one year after participating in the trial. The other two patients exhibited responses to the injection, as evidence by an increasing concentration of 5-FU; nevertheless, there were no clear indications of tumor regression. These two patients passed away approximately 100 and 200 days, respectively, after the trial.

The ability of the modified *Salmonella* to locally thrive and release CD enzyme within the tumor did not perform as effectively in the human body compared to the results observed in mice experiments. Nonetheless, the modified *Salmonella* strain still displays considerable potential for cancer treatment, and thus more effective modifications need to be explored to improve the ability of the strain to grow and release proteins within tumors [18].

Three clinical trials are still progressing: (1) Dr. Garin Liu using SGN1 strain to treat advanced solid tumor patients (2) Dr. Gerald Batist employing Saltikva strain for Metastatic Pancreatic Cancer (3) Dr. Alexander and colleagues using oral-administrated Salmonella as a carrier to deliver DNA vaccine to treat neuroblastoma [19]. In these new clinical trials, researchers have begun exploring alternative administration methods, such as oral delivery, and have experimented with various modifications to increase the bacteria's reliance on exogenous nutrients to improve its localization ability. Additionally, they have investigated different tumor-suppressing pathways in order to maximize inhibitory effects.

2. Quorum Sensing System

2.1. Introduction of Quorum Sensing System

The concept of controlling bacterial populations through their intrinsic sensing systems traces its origins back to the fermentation process like brewing wines. In these processes, as the bacteria proliferate and thrive in the low-oxygen environment, they metabolize glucose into alcohol and carbon dioxide. Thus, alcohol accumulates, eventually reaching levels that prove lethal to the bacteria, culminating in the successful production of sterile and high-quality wine. Brief illustration of the common mechanism of all quorum sensing system can be seen in figure 1.

Quorum sensing represents a more sophisticated mechanism by which bacteria regulate their colony size. Generally speaking, quorum sensing system enables bacteria to gauge the density of their population and coordinate collective actions to enhance or ensure the efficacy of these actions. The pioneering work of Kenneth Nealson's team unveiled the quorum sensing system in a marine bacteria known as *Vibrio Fischeri*. This bacteria employs the quorum sensing system of the "Lux family" to regulate the expression of luciferase, an enzyme that could make the bacteria glow, which in other words is its bioluminescence. Typically, *Fishceri* exist individually, resembling zooplankton. However, rather than living separately, they prefer to colonize in the mental cavity of the Hawaiian bobtail squid where they could hide from predators and get nutrition more easily. Like paying the rent, during this mutualistic relationship, the bacteria have to benefit the squid in return, which is lighting the upper part of the squid and thus concealing the squid from predators when observed from both beneath and above during stary nights. The Fischri would only emit light when residing within the squid. In order to determine their presence, they continuously produce a specific autoinducer molecule which automatically exports through diffusion into their surroundings as soon as they are produced. LuxI is the gene responsible for encoding this autoinducer. Once the autoinducer reaches a critical threshold, detectable by membrane-bound proteins on the bacterium's surface, it enters the Fischeri cell via transport proteins and binds to LuxR. This interaction forms a complex that initiates operon activity, subsequently triggering the transcription of LuxI, LuxA, and LuxB. LuxI will further produce additional autoinducers, creating a positive feedback loop. Simultaneously, LuxA together with LuxB would collaborate in the synthesis of luciferase, the enzyme responsible for the emission of blue-green light [20-22].

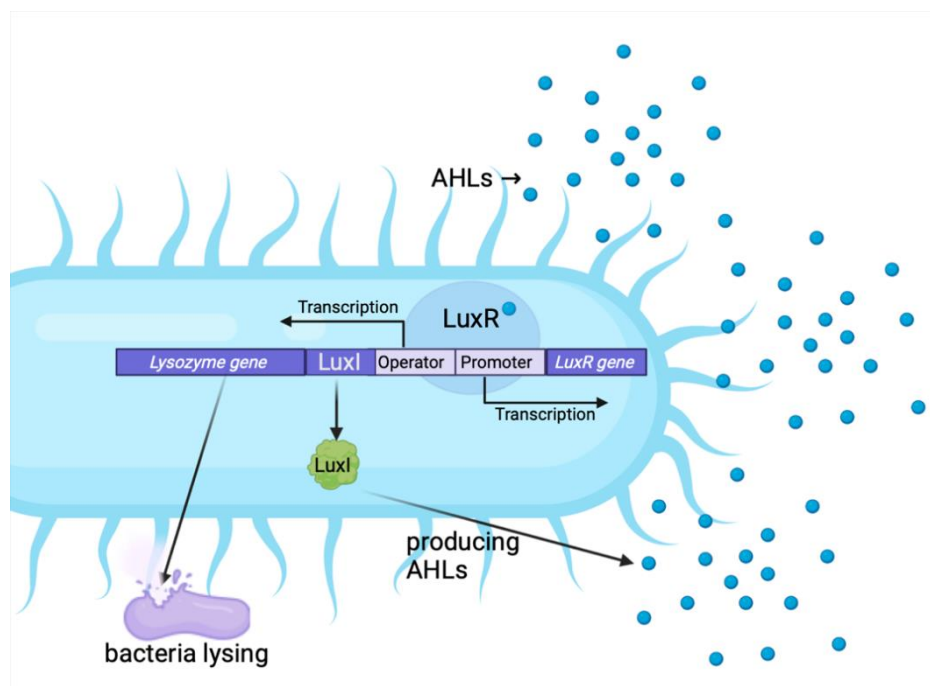


Figure1. The overall perspective of quorum sensing mechanism. Picture credit: original

The case of Fischri and the bobtail squid provides a quintessential illustration of how the quorum sensing system operates. In addition to this example, quorum sensing can lead to either expressing or inhibiting the expression of the downstream genes and therefore it could play many different roles in cell-mediated communication encompassing determining the appropriateness of launching a virulent attack, seizing opportunities to construct biofilms, and regulating motility, among other functions [23].

There exist numerous types of autoinducers in quorum sensing, and the associated pathways exhibit considerable diversity. It is accepted that the quorum sensing systems can be categorized into four primary groups based on the discernible autoinducers and their respective producing proteins: (1) LuxS, which produces AI-2; (2) LuxR/LuxI which produces AHL; (3) autoinducer peptides (AIPs); (4) AI-3/epinephrine/norepinephrine [12, 23-24]. However, there are also other quorum sensing systems that might exhibit similarities to or overlap with the aforementioned categories, and some have not been fully characterized, thus often omitted in conventional reviews of quorum sensing systems. Examples include the AI-1(CAI-1/LAI-1) system and its synthetase CqsA/LqsA, as well as the PAME (hydroxyl-palmitic acid methyl ester) and the DSF (methyl dodecenoic acid) [25-28].

2.2. Reviews on Four Main Quorum Sensing Systems

2.2.1. AI-2 and Enzyme LuxS System in *E. coli*.

The autoinducer-2 (AI-2) is produced in the methyl activation cycle (CAM) assisted by the LuxS enzyme. LuxS facilitates the conversion of its substrate, S-ribosylhomocysteine (SRH), culminating in the generation of homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which forms the chemical foundation of AI-2 as an array of isomeric variations. DPD itself is limitedly stable in aqueous environments like in the cellular cytoplasm, predisposing it to spontaneously cyclization into 4-hydroxy-5-methyl-3(2H)-furanone (HMF). Some cyclized isomers of HMF including S-DHMF and R-DHMF, assume roles akin to AI-2. These molecules may further undergo hydration processes, converting into other complexes like S-THP, S-THMF, and R-THMF. These complexes, too, contribute to the multifaceted dynamics of the AI-2 in the quorum sensing process [24, 27, 29].

To briefly summarize, the LuxS enzyme coming from the substrate SRH greatly helps the production of AI-2. The synthesis of SRH, in turn, emerges as a crucial component of the synthesis of AI-2 as well. Another enzyme, Pfs, additionally contributes to the transformation of SAH into SRH by catalyzing the cleavage of the glycosidic bond between MTA and SAH [23, 29].

After the generation of the AI-2, they could automatically permeate out of the bacteria rapidly and accumulate in the external environment. Conversely, the import of AI-2 into the cell is a regulated process, emphasizing the accumulation of the sufficient quantity of AI-2 for cellular uptake. In the periplasmic space of the *E. coli*, AI-2 forms a complex with LsrB traversing the bacterial membrane via transporter LsrC and LsrD. During this transport, the enzyme LsrA hydrolyzes ATP into ADP and a molecule of the phosphate group, providing the requisite energy for this import process. Upon entry into the bacterial cytoplasm, AI-2 binds to LsrK, a kinase enzyme, leading to its phosphorylation into AI-2-P which would subsequently bind to LsrR forming a complex. The LsrR and AI-2-P complex downregulates the expression of the *LsrACDEFG* operon and thus decrease the bacteria's ability to import the AI-2 while governing its ability to degrade AI-2-P, a product primarily executed by the LsrEFG proteins [22, 29, 30].

2.2.2. AHL and LuxI/R system. In the introductory context of the quorum sensing system, the *Vibrio fischeri* bacterium serves as an illustrative example, utilizing this mechanism to initiate its bioluminescence system. In this system, LuxI acts as the synthase of AHL while LuxR functions as the receptor protein for AHL. AHL molecule typically consists of two components: the hydrophilic ring homoserine lactone ring (HSL) and one hydrophobic acyl side chain. Therefore, the overall hydrophobic or hydrophilic nature of AHL depends on the length of its acyl side chain. AHL, owing to its HSL structure, can diffuse through the membrane passively, without the expenditure of energy. Thus, the diffusion of the AHL hinges on the concentration difference between the interior and exterior of the bacterial cell. [21, 24]

When the bacteria density is low, AHL production outpaces its accumulation, allowing AHL molecules to readily exit the cell, which leaves LuxR unbound within the cell, precluding its binding to the *PluxI* promoter. Conversely, at high bacterial densities, exterior AHL concentrations rise, impeding its diffusion out of the cell. It hence binds to the LuxR protein, forming a complex binding to the *PluxI* promoter, which would initiate the expression of the downstream gene including the LuxR gene and the *LuxICDABE* gene, collectively constituting the Lux protein family. The region housing these Lux protein-coding genes is referred to as the Lux box, and the precise role and location of the Lux box remain subjects of ongoing investigation within the scientific community.[20, 21, 24]

It is worth noting that, one special category of LuxR, called the orphan LuxR or the solos LuxR, possesses the ability to recognize AHL molecules produced by other bacterial species. Interestingly, the AHL from other strains can also initiate the expression of downstream genes, which suggests that the AHL and LuxI/R system may serve as a commonly employed system among all gram-negative bacteria, thereby representing an inter-species communication platform within the bacteria realm [20, 21, 31].

Given the variability in acyl side chain composition, a diverse array of AHL molecules exists, each capable of forming complexes with derivatives of LuxR and thereby governing vital bacterial functions. For instance, in *Vibrio*, the downstream *LuxICDABE* gene manages bioluminescence; In *Pseudomonas aeruginosa*, which utilizes two parallel AHL systems, distinct pairs of AHL bind to LasR or RhIR hence initiating the synthesis of hydrogen cyanide and alkaline protease; In *Chromobacterium violaceum*, AHL interacts with CviR, promoting the expression of the violacein pigment production. Importantly, when the side chain of the AHL exceeds a certain length, rendering the AHL no longer a micromolecule, the ability to diffuse through the membrane passively is deprived, converting the export into active transport mechanisms, which further expand the AHL and LuxI/R systems underscores the diversity and complexity inherent in these intercellular communication pathways [20, 21, 32].

2.2.3. Autoinducer Peptide (AIP) system. The AIP precursor is initially synthesized by the attached ribosomes. Subsequently, it undergoes posttranslational modifications, a process occurring during its excretion, to be activated and stabilized. During the excretion, the ATP-binding cassette transporter (ABC) will be responsible for facilitating the export of the AIP. Once modified, activated, and exported, AIP will accumulate in the extracellular environment. Similar to other quorum sensing systems, when the amount of the AIP reaches a physical critical concentration threshold, there is a reduction in further excretion.[28] Instead, it binds to a receptor on the outer membrane, causing the phosphorylation of the conserved histidine residue. This residue could further activate the receptor kinase which then transports a phosphoryl group to the aspartate residue of an intracellular response regulator which significantly influences the expression of various target genes, including the ABC transporter and others. The AIP system is a common feature observed in gram-positive bacteria.

2.2.4. AI-3/epinephrine/norepinephrine system. This system was reported in *E. coli*. where it regulates the toxins gene's expression. In a review written by Cristiano et al., they delve into the mechanism of AI-3 specifically in a type of *E. coli*. known as EHEC. In the human intestine, the resident flora produces AI-3 as signaling chemicals, while the human host produces epinephrine and norepinephrine to govern the intestinal activity. The QseC protein, located on the membrane of EHEC, could sense the accumulation of these chemicals and undergo autophosphorylate at precise intervals. Subsequently, it forms complexes with its response receptor, QseB, promoting QseB's phosphorylation. Another membrane-bound sensing protein in EHEC which is QseE interacts with epinephrine and norepinephrine and thus phosphorylates QseF protein. Alongside these Qse proteins, numerous other proteins are included in this phosphorylation cascade, ultimately resulting in the expression of LEE gene. The LEE gene is a significant gene for EHEC since it regulates the expression of the motility gene *flhDC*, the Shiga toxin gene *StxAB*, the effacing (AE) lesion formation, and the *QseEF* gene. Although AI-3 is not a direct regulator for the number of bacteria, it plays a crucial role in regulating the synthesis of toxins and orchestrating other meaningful collective activities, rendering it a component in quorum sensing systems the quorum sensing system demonstrating the significance of the system not only including the quantitative regulation but also encompassing various other facets of bacterial behavior [33, 34].

3. Prospective of Combining Quorum Sensing System with BMCT

3.1. Examples of Utilizing Quorum Sensing System in BMCT

Using anaerobic bacteria to release anti-cancer chemicals has been a popular topic for long. However, the clinical study of using quorum sensing system to precisely control the turn-on and turn-off expression of the desired chemicals is still under progress.

One trial done at MIT and Harvard Medical School in 2015 used Lux system to replace the original quorum sensing system in *Salmonella* to harness the specificity of the Lux system in *Fishiri* which is a member of marine plankton [35]. The Lux in *Fishiri* does not share with other bacteria strains in mammals so it allows the quorum sensing system to function independently without interrupting normal function of other resident bacteria in mammals. Additionally, the study combined the LuxI with green fluorescent protein (GFP) which produce 3OC6HSL, a kind of HSL, that activates and binds to LuxR. The compound formed from 3OC6HSL and LuxR can be recognized by the operon of LuxI so that positive feedback of 3OC6HSL production is established. The team chose direct injection to deliver the system, and follow-up detection of the concentration of bacteria in both tumor and the liver were conducted to monitor the bacteria diffusion. Another research where many genes are deleted to create a tryptophan auxotrophic *Salmonella* strain with reduced toxicity and improved invasion ability to immune system. These properties could facilitate bacteria to better locate into the tumor. The modified bacteria also carry vectors that code cytolysin-A (ClyA) which would induce apoptosis of tumor cells. Significant reduced tumor size indicates its promising perspective in treating human solid cancer [30].

3.2. Design of a novel potential BMCT strategy based on quorum sensing and *Salmonella* Typhimurium

3.2.1. Epidemiology and Necessity. Colorectal cancer (CRC) as a significant global health concern ranks third in terms of cancer incidence (6.1% of all cancer cases) and is the second leading cause of cancer-related mortality (9.2% of cancer deaths worldwide). According to data from 2020, the incidence of CRC even reached around 30% in Oceania and Europe, and the mortality rate has also risen to around 10% among all continents. The global burden for CRC is continuously growing, with approximately 2.2 million incidences and 1.1 million deaths. [36] As CRC are solid tumor that could be treated with the BMCT method by which *Salmonella* Typhimurium could be an ideal material. Through oral intake, the modified *Salmonella* could reach the nidus on the colorectal, reside there and proliferate.[37] After sensed sufficient amount of bacterial population through the quorum sensing system the bacteria would lyse and release the protein so the growth of the tumor would be inhibited. As the bacteria itself could be the pathogen that induce inflammation and recruit immune cells, the lysing process of the bacteria could also enhance the recognition of the immune cells to the tumor cells. Therefore, the ideal outcome for this treatment is to suppress the growth of the existing tumor and meanwhile improve the supervising ability of the immune system [14, 38]. A brief illustrative picture showing the design of the immune enhancer can be seen in the figure 2 below.

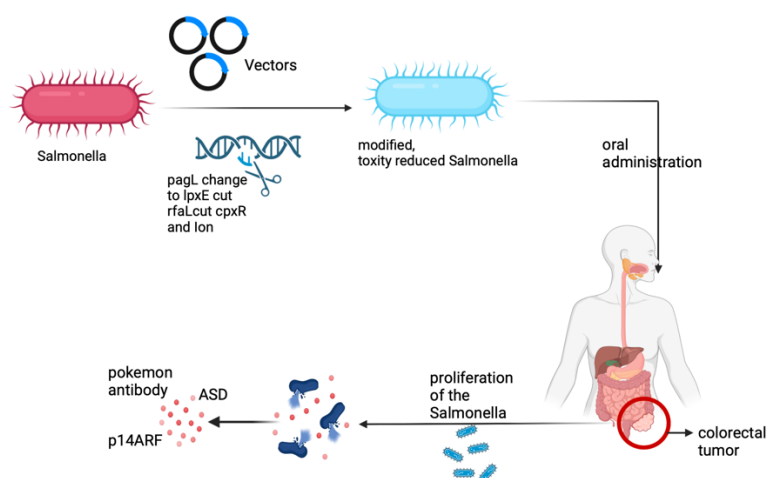


Figure 2. The outline of the design uses modified salmonella to treat colorectal cancer through oral administration. Picture credit: original.

3.2.2. Modification on Quorum Sensing System. The original quorum sensing system, the LsrR system, in *Salmonella* releases autoinducer 2 (AI2) in the environment and later absorbs them back into the bacteria through transport protein LsrA, LsrB, LsrC and LsrD while the AI2s reaches the threshold. However, instead of inhibiting the downstream gene, initiating a suicide gene would be more efficient. Therefore, here is where the LuxR quorum sensing system could step in. The LuxR quorum sensing system generates AHL as its autoinducer and the AHL-LuxR complex could initiate the expression of the downstream genes. The genes coding lysozymes are off when *Salmonella* density is low and would be turned on when the bacteria amount reaches the threshold [24, 39, 40].

3.2.3. Techniques and Establishment of the Vector. Vectors would be constructed through PCR and Gibson Assembly [41] to introduce desired genes into the *Salmonella*. The Pokémon antibody single chain variable fragment (scFv) gene, p14ARF, ASD and fragment from plasmid pET-N-His-TEV (from the Beyotime company) would be assembled to form the full vector which could be seen in figure 3.

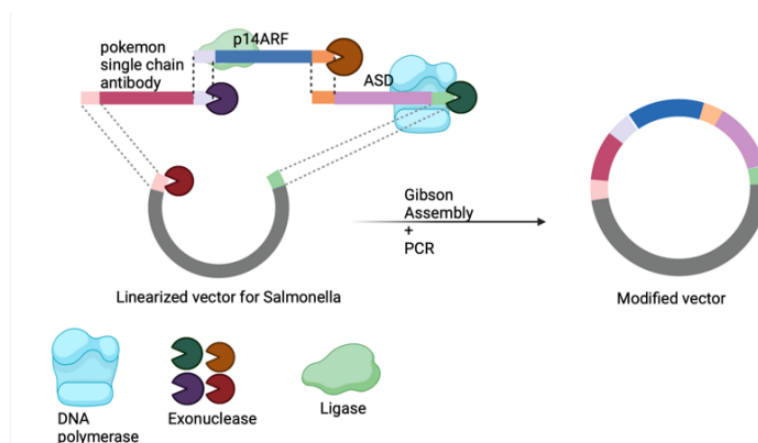


Figure 3. The establishment of the vector. Picture credit: original.

The rationale of using Pokemon antibody scFv and p14ARF gene is based on the discovery of the “Pokemon $\uparrow$ →ARF $\downarrow$ →MDM2→P53 $\downarrow$ ” pathway which has been shown to be responsible for the transition from benign colorectal tumor to a real cancerous malignancy. In order to invert the tumor growing process, high expression of P53 protein can be induced by binding Pokemon scFv with Pokemon protein to reduce its amount as well as increase the volume of p14ARF, so that cell cycle could suspend in the G1 phase. In detail, higher expression of p53 manifests in elevated levels of MDM2 and p21CIP1 with cell cycle arrest in both G1 and G2/M which are part of the interphase [42-43].

ASD gene codes for the enzyme ASD that produce DAP which is one of the most important components to form the cell wall of Salmonella. The insertion of ASD gene and the removal of the ASD gene from the strain genome puts Salmonella under certain survival pressure forcing them likely to retain the vector instead of lysing it. This modified Salmonella strain would be selected through cultivating them in agar plates without DAP [9, 37, 39].

Terminator, promoter and an operator integrated in the T7 expression system in Ecoli could ensure the self-replication of the vector and vectors be separated into two daughter cells during bacteria proliferation. To ensure the expression of the desired gene and the replication of the vector, the gene of the RNA polymerase called DE3 that could initiate the expression of the T7 promoter must be inserted into the genome of the bacteria [44].

To deliver the vector into the bacteria, positive charges need to be introduced since the negative phosphate group on the surface of the vector and the bacteria would repeal each other prohibiting the vector from getting into the bacteria. Calcium chloride solution is used to neutralize the negative charge. A temperature shock at 42 Celcius degree could open pores on the cell membrane, increase the cell permeability of the bacteria and allow vectors to enter. Cultivation of bacteria should be carried out at 37 Celcius degrees.

3.2.4. Animal Experiment. The hypothesis of the design for the animal experiment is as follows. The study with the animal model is divided into blank, control, and experiment groups. Mice in the blank group are without colorectal tumors and not injected with any vaccine or placebo.

The disease animal model for the control and experiment group is established by mutation of Apc, injection of DMH, and oral intake of the DSS. Apc is a crucial tumor suppressor gene within the Wnt pathway, and it plays an important role in the initiation and development of colorectal cancer. The 850th codon (TTG) of the Apc gene encoding leucine could be edited into a stop codon (TAG) to form the mutated gene [45]. Both positive and negative control groups would be established. For the positive control group, the disease model mice will be treated with Keytruda, a chemical shown to be effective in suppressing CRC tumor growth. Placebo will be provided to the negative control group. In

the experimental groups, aside from considering variations in bacterial concentrations within the BMCT or vaccine, it is essential to address the effective duration of this treatment.

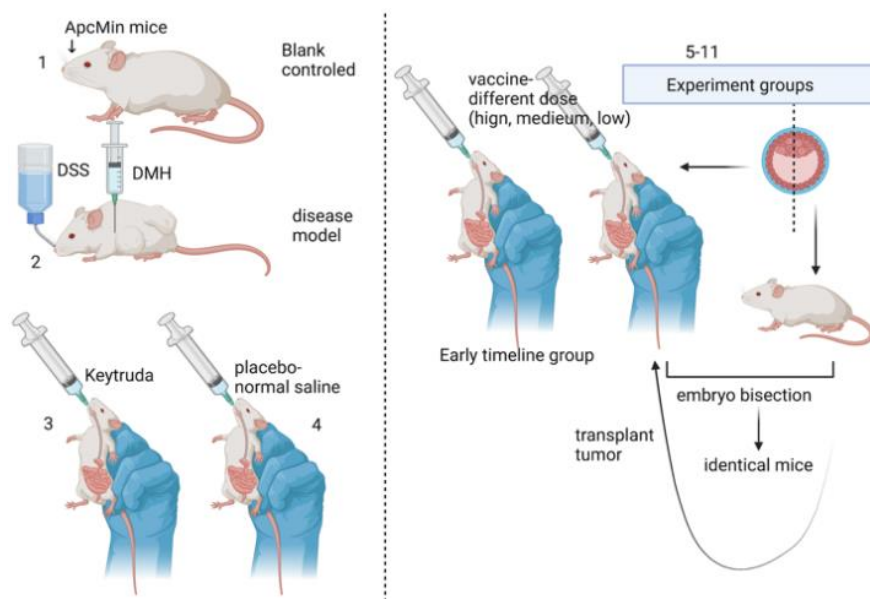


Figure 4. The group division and operation on mice in the animal experiment. Picture credit: original.

Consequently, the experimental groups can be categorized into two main groups: the typical experimental group and the lagging experimental group. Except for the different concentrations of the bacteria in the BMCT, the effective duration this treatment could induce is also an important concern. For the typical experiment group, we can gather data on the estimated time duration for the clearance of bacteria taken. Conversely, in the lagging group, a new tumor from the homologous mice could be transplanted to the experiment group after the elimination of bacteria to examine whether this treatment could stimulate an immune response or not. Designation of the animal experiment is illustrated in figure 4 above.

3.2.5. Medicine Delivery. Oral administration will be the optimal method to deliver bacteria to the target location primarily because it is the most prevalent way employed by previous researchers for administering the *Salmonella* Typhimurium vaccine carrier. Furthermore, there are additional reason that solidify our choice of this approach. In contrast to alternative methods of administration, the mice exhibit superior adaptability with less weight loss and extended average life span comparing to mice that are muscular injected or other delivery method. Besides, by this way of bacteria administration, most *Salmonella* will be absorbed in the jejunoileal vein within the small intestine and the bacteria are less likely to enter the brain, liver or other unrelated organs and tissues. These findings have been substantiated through histology analysis by Doctor Aganja and his research team [39-40].

4. Conclusion

In conclusion, this review begins with a brief overview of the development of BMCT, where doctor Coley is mentioned to explain that people initially noticed and harnessed the ability of bacteria to induce inflammation and thus to activate the immune reaction in the specific locations where tumors are. Subsequently, the review included and summarized more pros of bacteria in treating cancer and additionally emphasized the outstanding characteristics of *Salmonella* in cancer treatment. Several clinically ideal modified strains are also included in this review, which naturally transits the topics to the summary of clinical experiments using them to treat cancer. The second section of this passage mainly focuses on introducing the quorum sensing system which lays the firm foundation for making

BMCT a reality. It started with a case study where the marine bacteria, *Vibrio fischeri*, uses quorum sensing system to maintain its normal functions as well as the common management mode of the quorum sensing system. Next, according to the different types of autoinducer molecules and the enzyme responsible for the production of that autoinducer, the review details four systems while briefly summarizing other systems that have been discovered. In the third section, after introducing two distinct trials on mice indicating an ideal result on mice, a design, as an outlook of a possible treatment idea combining those two trials, is discussed in hopes of inspiring coming researchers in aspects that they could further their study.

Acknowledgement

To assist readers of this review in having a better understanding of the classification of autoinducers, the following reviews are highly recommended: the annual review from Wai-Leung Ng and Bonnie L. Bassler, the article “Emerging applications of bacteria as antitumor agents” from Vipin Chandra Kalia et al., and the article “Genetically engineered *Salmonella* Typhimurium: Recent advances in cancer therapy” by Liang et al.. Also, the concept of the sensing protein for the threshold that all autoinducers could achieve poses challenges in its clarity as the potential mechanisms remain unresolved, interpretation of these mechanisms in this review is limited to a macro-scale perspective without detailed chemistry. The potential and promise of employing quorum sensing systems in cancer treatment offer a less intrusive therapeutic approach. The author deeply appreciative of the invaluable support and contributions from Jiahua Ge, Yilin Yin, and Ruoxi Liu in shaping the design of the vaccine discussed in this review. Lastly, I extend my heartfelt gratitude to all the teachers who have provided invaluable guidance along my research journey. Their support has been instrumental in enabling me to conduct research, compile references, and write this review independently.

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Assessing risk factors for diabetes in women: A logistic regression analysis

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Abstract. The prevalence of diabetes has become an increasingly pressing health challenge, with women emerging as a particularly affected demographic. As global studies underscore, the incidence of diabetes has been growing at an alarming rate, leading to substantial health consequences and creating economic burdens for healthcare systems. To address this, the research turned to an in-depth analysis using logistic regression. The data, sourced from the National Institute of Diabetes and Digestive and Kidney Diseases, encompasses findings from 769 individuals updated as of 2022. This comprehensive dataset incorporates diverse variables, from Pregnancies and Glucose levels to more specific markers like the Diabetes Pedigree Function. Intriguingly, the analysis revealed a dominant age group between 20 and 40 in the dataset. In terms of correlations, variables such as Glucose, BMI, Age, and Pregnancies displayed strong positive associations with the presence of diabetes. These findings not only corroborate existing medical knowledge but also shed light on potential risk determinants previously underemphasized. However, it's essential to approach these findings with caution, acknowledging the limitations inherent in the dataset's scope. Despite these constraints, the significance of this research remains profound. By emphasizing both familiar and overlooked factors, this study paves the way for more targeted and effective early interventions, ultimately aiming to improve the prognosis and quality of life for diabetic patients.

Keywords: Diabetes, risk factors, logistic regression.

1. Introduction

Diabetes has been a long-lasting health issue among many people, especially diabetes has become the most predominant phenomenon among women. Recent studies have shown that diabetes prevalence has been on the rise, with significant health and economic impacts worldwide [1]. In particular, the prevalence of diabetes has been notably increasing in several countries, with global estimates suggesting that the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and is projected to rise to 4.4% in 2030 [2]. Another study indicated that the global burden of diabetes had significantly increased since 1990, with an incidence of 22.9 million and a prevalence of 476.0 million in 2017 [3]. Furthermore, specific regions such as Southeast Asia have seen a rapid increase in diabetes cases [4]. In Indonesia, urban childhood residence and high education have been identified as risk factors for early-onset adult diabetes [5]. Moreover, there's a significant prevalence of diabetes among patients with pulmonary tuberculosis, emphasizing the comorbidity of these conditions in global health scenarios [6]. Recent data from the US also highlighted that the age-standardized prevalence of diabetes increased

significantly from 9.8% in 1999-2000 to 14.3% in 2017-2018 [7]. The factors involved in the development of lung cancer were very complex, and its occurrence was the result of multi-factorial, multi-gene, and multi-step interactions [5]. Domestic and foreign scholars had found some correlation between lung cancer and smoking [6], passive smoking [7], and sleep duration [8, 9]. In addition, Sun found that other factors also had some impact on lung cancer [10]. However, this literature studied fewer lung cancer factors and lacked systematic and complete statistics. Therefore, this paper focuses on 15 factors (Gender, Age, Smoking, Yellow fingers, Anxiety, Peer-pressure, Chronic Disease, Fatigue, Allergy, Wheezing, Alcohol, Coughing, Shortness of Breath, Swallowing Difficulty, and Chest pain) to study whether they have any effect on lung cancer, and further select an appropriate model to study the degree of correlation between these factors and lung cancer.

After returned the manuscript must be appropriately modified. Hence, it's crucial to delve deeper into the factors associated with diabetes to facilitate early detection, treatment, and prevention, thereby reducing associated morbidities and mortalities. This paper aims to explore various potential factors that could lead to diabetes, assisting individuals in assessing their risk and adopting preventive measures accordingly for females.

The factors contributing to diabetes are multifaceted, encompassing genetic, environmental, and lifestyle elements [8]. Numerous studies have highlighted associations between diabetes and factors such as obesity [9], sedentary lifestyle [10], and dietary habits. Nevertheless, much of the existing literature has focused on a limited set of factors, often lacking comprehensive statistical analysis on a particular group: Women. Thus, this study will concentrate on the parameters provided in the dataset, namely Pregnancies, Glucose, Blood Pressure, Skin Thickness, Insulin, BMI, DiabetesPedigreeFunction, and Age, to ascertain their potential influence on diabetes.

In line with this research focus, various scholars have adopted different analytical models. For instance, they have been using Decision Trees, Random Forest, and Naive Bayes to perform forecasts and analysis on existing dataset on diabetes. However, it would only be performing logistic regression on the existing data set to help females to prevent and improve diabetes in advance.

2. Methods

2.1. Data Sources

The data for this literature is collected from the Kaggle website, which was compiled by AKSHAY DATTATRAY KHARE. This dataset is originally from the National Institute of Diabetes and Digestive and Kidney Diseases and updated in 2022 for 769 individuals.

2.2. Variable Selection

The data used in this analysis counts a total of 768 people, including those who have and do not have diabetes. The patients' ages range from 21 to 81 years. The data contains 8 variables: Pregnancies, Glucose, Blood Pressure, Skin Thickness, Insulin, BMI, Diabetes Pedigree Function, and Age.

Note that for this table 1, "Number1" represents the total number of people in the dataset (which is 768 for all variables). "Diabetes1" represents the number of people with diabetes who have non-zero values for the respective variable.

Table 1. This caption has one line so it is centered.

Elements	Logogram	Number1	Diabetes1
Pregnancies	x1	768	230
Glucose	x2	768	266
Blood Pressure	x3	768	252
Skin Thickness	x4	768	180
Insulin	x5	768	130
BMI	x6	768	266
Diabetes Pedigree Function	x7	768	268
Age	x8	768	268

Table 2. Age distribution and diabetes prevalence by age group.

Age	Number 2	Diabetes2
[21-30]	396	84
[31-40]	165	76
[41-50]	118	65
[51-60]	57	34
[61-70]	29	8
[71-80]	2	1
[81-90]	1	0

Note that “Number 2” represents the number of people in each age group. “Diabetes 2” represents the number of people in each age group who have diabetes. The majority of people in the dataset are aged between 21-30 and 31-40 years old (Table 2).

3. Results and Discussion

The majority of the individuals in the dataset are between the ages of 20 and 40, meaning there are fewer older individuals compared to younger ones (Figure 1). The dataset primarily captures data from relatively younger individuals. This might be a reflection of the sample population from which the data was collected. A right-skewed age distribution suggests that diabetes screening or the particular study from which this data originates may have had a higher representation of younger adults. It’s essential to be aware of this distribution, especially when considering factors like age-related risk. Younger individuals might have different risk profiles compared to older individuals.

Glucose, BMI, Age, and Pregnancies show the most substantial positive correlations with the Outcome (presence of diabetes). Features like BloodPressure and SkinThickness have weaker correlations with the outcome. Correlation coefficients provide a measure of the linear relationship between two variables. A value closer to 1 indicates a strong positive relationship, while a value closer to -1 indicates a strong negative relationship. The significant positive correlation of Glucose with the outcome reinforces the medical understanding that elevated blood glucose levels are a key indicator of diabetes. While BMI and Age also have positive correlations with the outcome, it’s crucial to note that correlation doesn’t imply causation. Higher BMI might be a risk factor for diabetes, but it’s one of many potential factors. Weaker correlations for features like BloodPressure suggest that, while they might play a role in the overall health profile, they might not be as directly indicative of diabetes as Glucose or BMI (Figure 2).

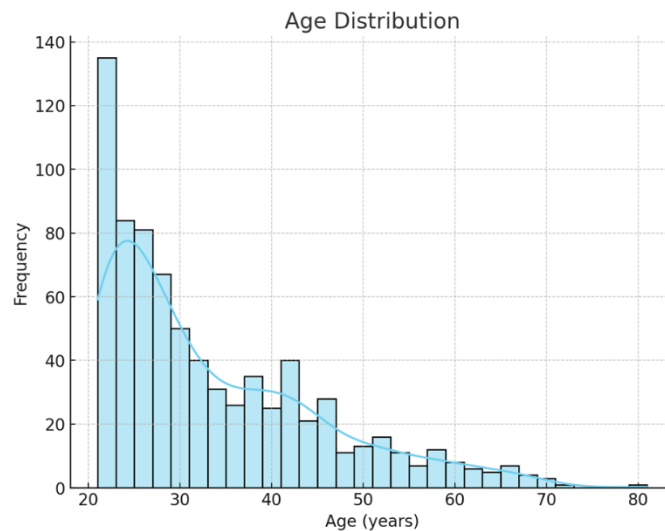


Figure 1. Age Distribution (Histogram).

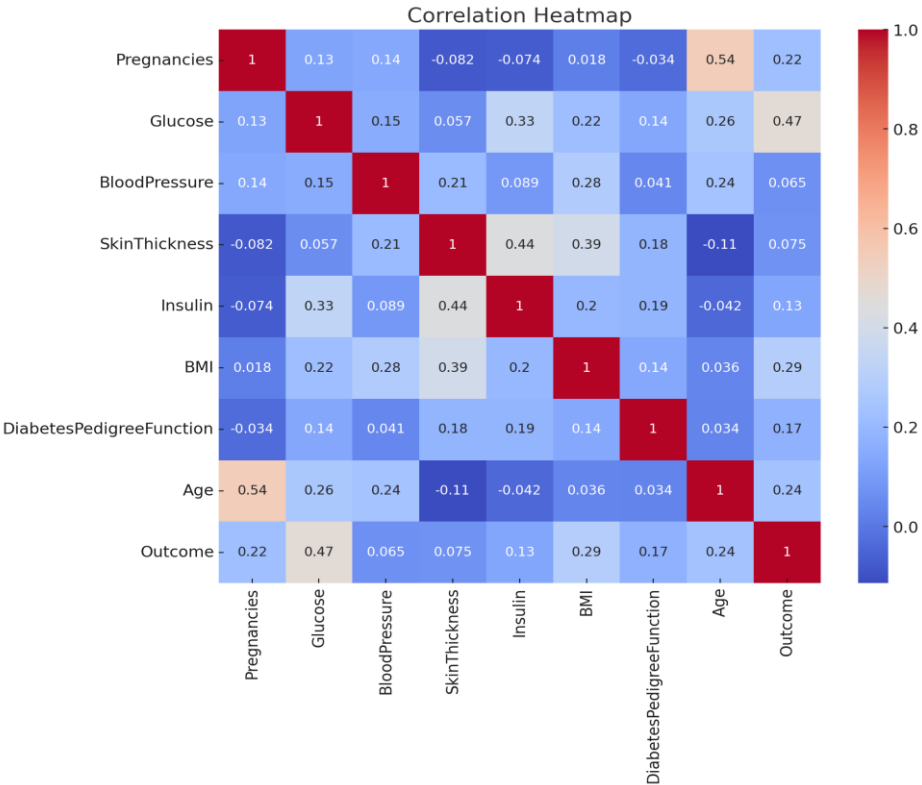


Figure 2. Correlation Heatmap.

Individuals with diabetes generally have higher glucose levels than those without. There’s a clear distinction in the median glucose levels between the two groups. Elevated glucose levels are a hallmark of diabetes. The body’s inability to regulate glucose (either due to insufficient insulin production or insulin resistance) leads to higher blood glucose concentrations. The boxplot visually reinforces this understanding, showing that even the lower quartile of glucose levels for diabetic individuals is higher than the median for non-diabetic individuals. However, there’s some overlap, indicating that while glucose is a strong indicator, it’s not the sole determinant. Some individuals might have higher glucose levels due to reasons other than diabetes (Figure 3).

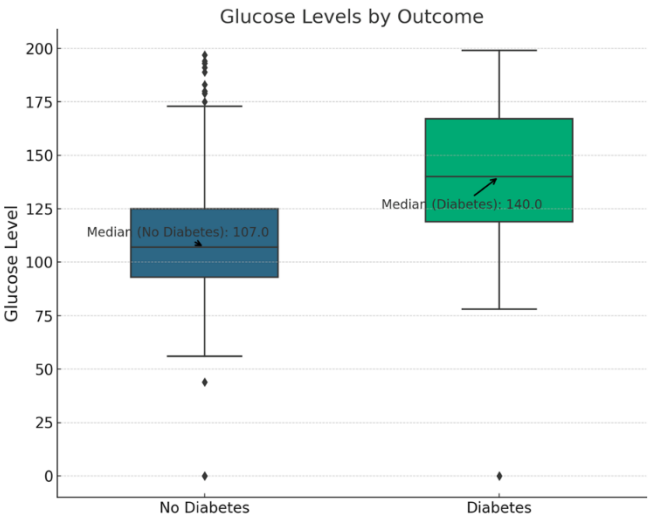


Figure 3. Glucose Levels by Outcome (Boxplot).

A general trend suggests that individuals with higher BMI values and higher glucose levels are more likely to have diabetes. However, there's a spread, indicating variability. Both elevated glucose levels and higher BMI values are recognized risk factors for diabetes. This plot visualizes their combined potential impact. While there's a general trend, the scatter and overlap between the two groups (diabetic and non-diabetic) suggest that these two factors, while influential, aren't absolute determinants. Other factors, both measured and unmeasured, play a role in the disease's onset. BMI, while a useful metric, is a broad indicator and doesn't account for factors like muscle mass vs. fat distribution, which can influence diabetes risk (Figure 4).

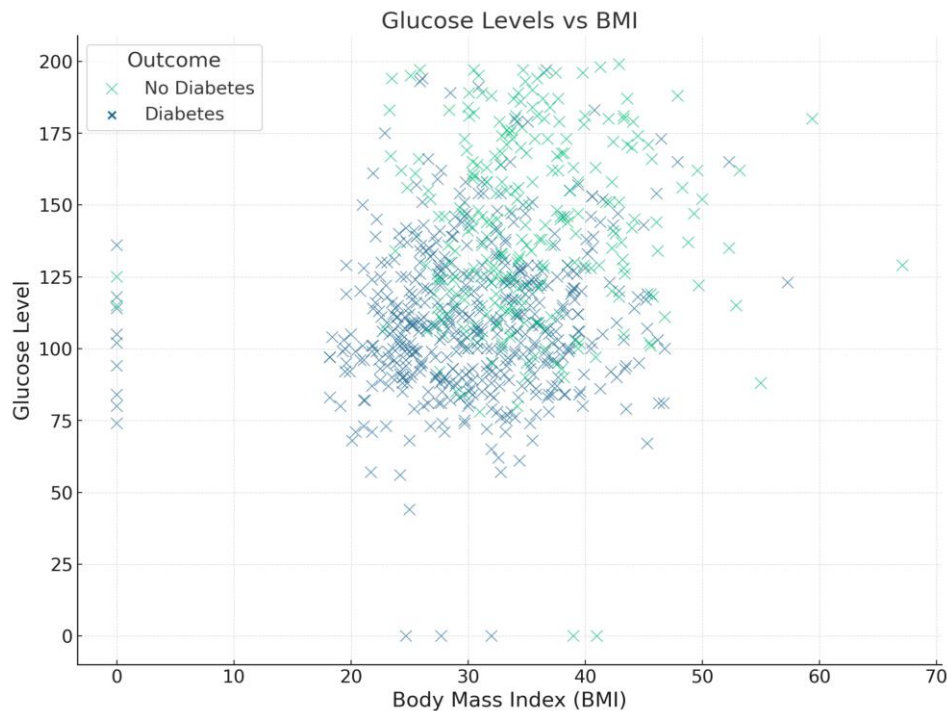


Figure 4. Glucose Levels vs BMI.

4. Conclusion

The present analysis delves deep into a diverse dataset, concentrating on potential determinants linked with the onset of diabetes, particularly in women. From the data, it was surmised that the likelihood of developing diabetes is potentially associated with factors like Glucose levels, BMI, Age, Pregnancies, Insulin, and the Diabetes Pedigree Function, some of which might have been underemphasized in earlier studies. While the study's insights are significant, it's imperative to note its limitations. Owing to the finite data scope, there could be inaccuracies in the model, and the sample doesn't encapsulate all age groups and ethnicities, potentially affecting the results' precision. Yet, the merits of this research are undeniable. The study adopts a pioneering methodology, amalgamating visual tools for an intuitive understanding and employing logistic regression, ensuring a holistic analysis compared to traditional single-factor methods. More than the well-documented factors like Glucose and BMI, the analysis brings to the fore possibly overlooked aspects like the Diabetes Pedigree Function. The implications of these findings could be paramount for future research, focusing on early detection and intervention, thereby elevating the quality of life and prognosis for diabetic patients.

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Deep learning-based image classification of MRI brain image

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Abstract. This article reviews the latest research on MRI brain image classification techniques based on deep learning. Firstly, MRI brain image classification and traditional machine learning methods applied to MRI image classification were briefly introduced. Then, a review was conducted on existing MRI brain image classification methods based on deep learning. The article reviews past and recent related research and provides a detailed introduction to the application of deep learning methods in MRI brain image classification. This includes some traditional machine learning methods and research achievements in deep neural networks (DNN), convolutional neural networks (CNN), and transfer learning. The most commonly used deep learning architecture for image classification is CNN. Research has shown that deep learning methods have high accuracy and performance in MRI brain image classification and can automatically extract image features for effective classification. Therefore, deep learning methods provide doctors with more comprehensive information, help them make more accurate diagnoses and formulate treatment plans, and have broad prospects for application in MRI brain image classification. In the future, the further development of deep learning technology will achieve better auxiliary effects on diagnosis, treatment, and scientific research related to the brain.

Keywords: deep learning, machine learning, MRI brain image, image classification

1. Introduction

With the development of technology, medical imaging technology continues to improve. Medical imaging technology includes various technologies, such as X-ray and Magnetic resonance imaging (MRI), playing an increasingly important role in clinical diagnosis and treatment. Brain imaging is a key task with broad application prospects in medical imaging, which can help scientists and doctors gain a deeper understanding of brain structure, function, and activity through different imaging techniques, thereby promoting the development of disease diagnosis, treatment, and brain science research. Among various imaging techniques, MRI is the most commonly used technique for brain imaging. MRI is widely regarded as the main brain imaging technique for assisting in diagnosing and treating brain lesions such as brain tumors due to its non-invasive nature and ability to generate high-quality brain image [1].

However, the traditional method in medical images field of manually selecting features relies on a large amount of professional knowledge and may result in some information in the image being ignored due to insufficient expression ability [2].

The concept of deep learning is an important research field in the field of machine learning. In recent years, deep learning has received increasing attention from relevant researchers. Applying deep learning method can find the internal structure of the data and discover the true relationship between data [3]. This demonstrates its ability to sensitively and effectively find hidden information and features in a large amount of data. Deep learning algorithms have important and widespread applications in audio recognition, image and video recognition, information retrieval, and related fields [3]. The development of deep learning also contributes to the recognition, classification, and quantification of patterns in medical images [4]. In medical image processing, the application of deep learning can improve the efficiency and accuracy of diagnosis, and fully express important information in medical images. Due to the ability of deep learning to accurately analyze a large amount of data, compared to manual work, this approach can extract more information from images and provide new developments in the medical field. Introducing deep learning into MRI brain imaging classification can also provide doctors with more comprehensive information, thereby assisting them in making more accurate diagnoses and providing treatment plans [1]. Therefore, the research on MRI brain imaging classification based on deep learning is very valuable and has received widespread attention.

This article reviews and summarizes the recent MRI brain image classification techniques based on deep learning. Firstly, a brief introduction was given to the MRI brain image classification and traditional machine learning methods applied to MRI image classification. Then, existing MRI brain image classification methods based on deep learning were reviewed.

2. Traditional machine learning methods of MRI brain image classification

In MRI medical imaging, due to various influences on the imaging process, such as tissue movement and artifacts caused by metal implants in the body, medical images inevitably have characteristics such as blurred edges of various tissues and uneven regional grayscale [5]. These will bring many difficulties to correctly classifying MRI brain imaging categories. In traditional machine learning, many different methods are applied to solve the classification problem of brain MRI images. Feature extraction and feature classification are the two main parts of machine learning for classifying MRI images. In addition to the feature extraction section, where researchers choose the feature extraction method for MRI images, different methods will also construct different classifiers. The algorithms or models they use to classify input data are different.

Support Vector Machine (SVM) is a classic classifier performing well in sample, high-dimensional, and nonlinear sample learning [5]. SVM has also been proposed in imaging to classify MRI images. In a 2013 study of Rosy Kumari, SVM was considered to have better accuracy and performance than other classifiers and was therefore selected for classification of MRI brain images [6]. Researchers used SVM to classify normal and abnormal brain images, as shown in Figures 1 and 2 [6]. This study used the Gray-Level Co-occurrence Matrix (GLCM) method to extract statistical texture features. This method can capture texture, structure, and spatial features by statistically analyzing the relationships between each pair of pixels in the image. SVM is a supervised learning based binary classifier that can construct a hyperplane in a high-dimensional feature space that can be used for classification. The classification stage of the study is divided into training and testing stages. During the training phase, brain image images were used to separate the feature attributes of image features, and separate and unique descriptions were provided for each classified category. In the testing phase, these feature space partitions created during the training phase are used to classify the image features of new brain images. The test results of this study indicate that the SVM method for MRI image classification has relatively high sensitivity, specificity, accuracy, and efficiency. By using the most effective kernel function, the accuracy of classifying normal and abnormal can reach 98.45% [6].

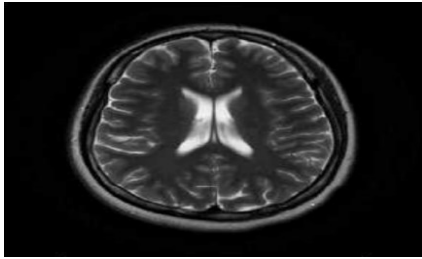


Figure 1. Normal MRI brain image [6].

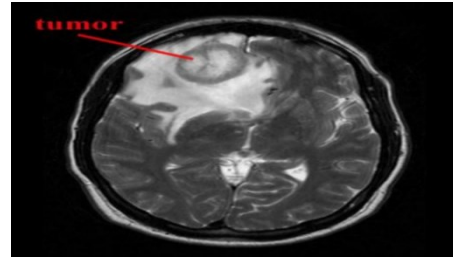


Figure 2. Abnormal MRI brain image [6].

In an intelligent classification study of Alzheimer's disease, researchers such as Zheng Yao used multiple machine learning classifiers. This includes using SVM based on paired classification to achieve intelligent diagnosis of AD. In these studies, researchers gradually optimized from a simple SVM binary problem to a five class problem, achieving an accuracy of 0.71 in the diagnosis of Alzheimer's disease [7].

Random forest is a combination classifier in machine learning, which can also achieve high accuracy in classification. In a previous Zhan Shu et al. study, random forests were used for MRI brain image classification [5]. In terms of feature extraction for brain MRI images, this study takes texture, grayscale, histogram features, Otsu threshold, and shape features [5]. The diversified feature extraction in this study can enrich the expression of image features. The core idea of random forests is to integrate multiple weak classifiers to achieve accurate classification results. After multiple experiments, the optimal coefficient of the model was obtained. The accuracy achieved with this coefficient is 0.941 [5].

In the study by Zheng Yao et al., the random forest method was also used for MRI brain image classification to diagnose Alzheimer's disease, but the accuracy obtained was 0.69, slightly lower than the SVM method [7].

However, researchers still raised a problem: In the training stage of machine learning, the prior knowledge of class labels of some pixels in the image needs to be classified first, which hinders the practical application of the random forest algorithm [5]. This issue has also become a follow-up research direction, including the researchers of this study [5]. Nowadays, this focal problem at that time can be solved through the application of deep learning. Nowadays, this focus issue can be solved through the application and development of deep learning technology, as deep learning has a certain degree of automatic feature extraction ability.

3. Deep learning based image classification of MRI brain image

Based on machine learning, deep learning is a special machine learning method that mimics the neural network structure of the human brain and learns and processes data through multi-level neural networks. Compared to traditional machine learning, deep learning uses deeper neural networks that learn complex relationships between data, which enables it to demonstrate stronger capabilities when dealing with complex data and problems. To some extent, deep learning can automatically extract features from data or images, and designing feature extraction can help further improve the performance of deep learning models. In recent years, more and more researchers have utilized the ability of deep learning to simulate more complex nonlinear relationships and its excellent performance, creating the current best solutions for many problems in medical image analysis applications [8].

There are different architectures for deep learning. DNN is widely used for classification or regression [8]. This method has also been widely applied in various fields and has achieved success in recent years. In the study by Heba Mohsen et al., deep neural networks were applied to classify brain tumors in brain MRIs [8]. In this study, certain feature extraction methods were still used. The research aims to apply deep learning techniques to classify brain MRIs and distinguish between normal brain and certain types of brain tumors, such as metastatic bronchial cancer tumors,

glioblastoma, and sarcoma, and measure classification performance based on the obtained results. The dataset used in this research contains 66 real human brain MRIs, including normal and abnormal images. The three types of brain tumors mentioned above appear in abnormal images. In the research process, the fuzzy C-means image segmentation technique was used to divide the brain MRI image into five parts: gray matter, white matter, cerebrospinal fluid, and skull and tumor tissue. The segmented tumor image is used for subsequent classification. Afterwards, due to the advantage of discrete wavelet transform in extracting the most relevant features in different directions and scales [8], this method was used for feature extraction of brain tumor images. This study uses deep neural network (DNN) to classify the generated feature vectors in the classification step. Using a 7-layer hidden layer structure of DNN and 7-fold cross validation technology for construction and training, its performance was evaluated on multiple performance indicators such as average classification rate, recall rate, accuracy, F-measurement value, and area under ROC curve. The results indicate that DNN classifiers perform well in all performance metrics compared to other classifiers. The accuracy reaches 96.97%, higher than traditional classifiers [8]. The results of this study emphasize the excellent performance and potential of DNN and deep learning in classification tasks.

CNN are a deep learning model widely used in medical image processing, and are most widely used in the classification of MRI brain images. In the study of Wadhah Ayadi et al., a new deep convolutional neural network (CNN) model was proposed for multi class classification of MRI brain tumors [9]. In this study, manual feature extraction was no longer necessary and automation was achieved. The general architecture of the proposed sequential model is shown in the figure 3.

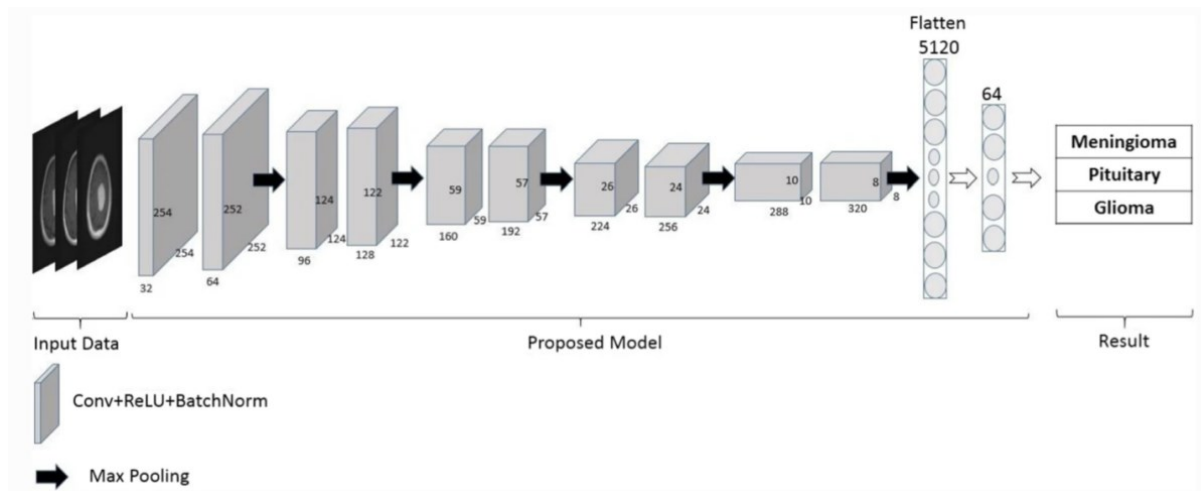


Figure 3. General architecture of sequential models [9].

The model consists of a 10 convolutional layer to extract features from MRI images, as shown in Figure 3 [9]. Because the complexity of CNN models increases with the increase of input size, researchers propose that reducing size can be used to minimize time complexity. The researchers used three common datasets to validate the performance of the model. The research results show that the model achieved the highest accuracy in MRI brain tumor classification. Compared to previous technologies, this model overcomes the difficulty of manually extracting features and achieves automation by using minimal preprocessing. The experimental results indicate that the model still has high effectiveness even in small datasets. The researcher's paper also mentioned that the model's structural selection and hyperparameter adjustment are effective for processing MRI images and obtaining more accurate results. In addition, this model has broad application prospects and can be used for other medical image classification, with strong application robustness.

Transfer learning is also effective in deep learning MRI brain image classification. In a study by Muhammad Fayaz et al., the CNN architecture was pre-trained on a large labeled dataset (ImageNet) using transfer learning to automatically analyze brain MRI images in Alzheimer's disease prognosis

[10]. In this study, researchers used a CNN architecture called AlexNet, which was trained on the ImageNet dataset. Through transfer learning, researchers transferred low-level features from the AlexNet model to the Alzheimer's disease classification task. This application of transfer learning can accelerate the learning speed of the model. In transfer learning, pre-training is conducted on a large dataset, and then the learned feature knowledge is transferred to the target task to improve the classification ability of the target task data. This step played a crucial role in this study. It enables CNN models trained on large datasets to exhibit higher accuracy and performance in medical image classification tasks. Enable researchers to transfer knowledge learned from natural images to medical image classification, thereby improving the accuracy of detection and analysis of Alzheimer's disease. After applying transfer learning, researchers used features learned from the ImageNet dataset for multi class classification of Alzheimer's disease, including subjects in normal, mild dementia, and moderate dementia stages. The classification results indicate that the system performs best in multi class classification of unsegmented images, with an accuracy of 92.8%. This method has achieved high accuracy in classifying test subjects as having Alzheimer's disease or not. This study has achieved significant results in the classification of Alzheimer's disease using MRI brain imaging, achieving an efficient automated system and providing an effective and accurate method for the classification and prognosis analysis of Alzheimer's disease.

A fundamental drawback of CNN is its lack of interpretability for its output [11], which is necessary to improve the model's reliability. In Shakila's study, an MRI dataset was used to train a 3D-CNN model to distinguish between cognitively normal individuals and Alzheimer's disease (AD) patients [11]. This study combined genetic algorithms and interpretability methods to extract important brain regions that significantly impact AD diagnosis. The study first used genetic algorithms and backpropagation-based interpretability methods to generate an occlusion map mask, and then improved it through an iterative process. Finally, the study obtained a brain mask from an AD patient composed of important brain regions. To verify the effectiveness of these extracted regions, the study compared them with expert research in the field, and the results showed that the model achieved an acceptable accuracy of 87% in 5-fold cross validation. Afterwards, the study used an unobstructed dataset with 96 different brain regions, trained a 3D-CNN model with an accuracy of 96%, and used it in the genetic algorithm stage to generate appropriate brain masks. Finally, using the interpretability method based on backpropagation, the study achieved a validation accuracy of 93% in 29 brain regions. This study extracted important brain regions of AD patients by combining genetic algorithms and interpretability methods, and demonstrated the effectiveness of this method. By improving the interpretability and accuracy of the model, research has contributed to the automatic diagnosis of AD.

Hossein Mehnatkesh et al. proposed an improved ant colony algorithm (IACO) to optimize the hyperparameters of classification algorithms and chose to use ResNet architecture [12]. To improve the accuracy of brain tumor classification, researchers first preprocess the images and select the most suitable pre-training model for the dataset. The researchers compared seven pre training structures and seven optimization methods, and selected ResNet and IACO, which had the best performance on the dataset, as the final models and optimization algorithms. Compared with other methods, the research results show that the proposed method has high performance and reliability. Finally, the model with the best hyperparameters was obtained. A new evolutionary strategy was introduced to solve the hyperparameter optimization problem of deep learning algorithms, and an improved ant colony algorithm (IACO) was proposed based on the most effective ant colony algorithm (CAO) among the original meta heuristic algorithms to improve the convergence speed and accuracy of the algorithm. This method has been experimentally validated and achieved high accuracy in brain tumor classification, reaching an accuracy of 99.02%, the highest among the seven meta heuristic algorithms. However, implementing this method is relatively time-consuming and requires further development of parallel algorithms to improve efficiency. This study provides a fully automated brain MRI image classification system that utilizes deep learning and evolutionary algorithms to obtain the optimal hyperparameters and model structure, which has good reliability in brain tumor classification of MRI images.

4. Conclusion

In conclusion, the MRI brain image classification method based on deep learning has important application value and broad prospects in the medical field. Compared to traditional machine learning methods, deep learning utilizes deeper neural network structures to learn complex data relationships, effectively extracting features automatically and achieving significant results in Brain MRI Image Classification. In MRI brain image classification, researchers have applied different deep learning models and methods, such as CNN, DNN, and transfer learning, to improve the accuracy and performance of classification. DNN have shown promise in their ability to automatically extract intricate features from MRI images, reducing the burden of manual feature engineering. It can be found that CNN is the most commonly used model architecture for computer vision tasks such as image classification. It has become the workhorse for MRI brain image classification, exhibiting remarkable accuracy and performance. Transfer learning has also played a pivotal role, allowing the transfer of knowledge learned from large datasets to enhance the classification of brain images. At now, deep learning-based MRI brain image classification has already made significant strides in improving diagnosis and treatment planning. It has the potential to revolutionize the field of medical imaging by providing more accurate, efficient, and interpretable tools. Looking ahead, the future of MRI brain image classification based on deep learning holds tremendous promise. As technology continues to advance, the future of MRI brain image classification is bright, promising better outcomes for patients and further advancements in brain science research.

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Research progress of wearable temperature sensor based on electronic skin

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Abstract. The rapid development of science and technology has led to an increasing demand for new sensors, among which wearable temperature sensors based on electronic skin have played a particularly important role in artificial limbs and intelligent robots. This paper introduces the physical structure and working principle of wearable temperature sensor based on electronic skin. In this part, it focuses on two models of electrical signal changes caused by temperature changes. At the same time, the circuit structure and signal transmission and signal processing mode are also mentioned. This paper also introduces the characteristics of the material used in order to achieve direct contact with the human body. Some researchers' work is also introduced, including accurate and linear reflection of temperature changes, fast response, durable use, and resistance to external interference, etc. The researchers' research on the realization of the above functions is mentioned in the paper. Finally, the practical application and future prospect of this type of temperature sensor are introduced. In addition, some more mature technologies for the preparation of such wearable temperature sensors based on electronic skin are also described in this paper.

Keywords: Temperature, Biosensor, Thermal Resistance, Electronic Skin.

1. Introduction

With the development of science and technology, more and more various types of sensors have entered the public eye, and wearable biosensors are one of the most popular types.

Compared with traditional analytical methods, the new biosensor detection device is smaller and more targeted, It is easy to realization of automated analyzing and detecting, detecting results with high precision, highly sensitive, quick in detecting and analyzing, low detecting cost, and continuous on-line detection [1].

Biosensors are so powerful that they can be useful in many fields. For example, in order to help humans or replace humans to perform some dangerous, high-risk tasks (such as handling nuclear pollutants), intelligent robots are made, in order to adapt to the environment and respond to changes in the outside world, they need to be similar to human perception, biosensors can play a role in order to achieve the above functions. Similarly, for some people with physical defects, out of humanitarian considerations, they need to live like normal people, so some auxiliary devices are developed, such as artificial limbs, people equipped with artificial limbs need the same perception and control ability as ordinary people, biosensors can help realize these functions.

The various types of devices mentioned above need to have the ability to accurately sense temperature. Temperature is an important indicator of the external environment, is also an important indicator of the state of the human body, people can judge whether it is suitable for some work at this time through the temperature, for the biosensor used in the human body, temperature measurement is more important. Body temperature can reflect many basic health conditions of the human body, such as the physiological activity of some tissues, organs and systems in the body, emotional changes and basic metabolism. Therefore, researchers have conducted a lot of exploration and research in order to design more advanced wearable temperature sensors, and they believe that temperature sensors based on electronic skin can play a good role. Therefore, they investigated many aspects of the design of the wearable temperature sensor based on the electronic skin, including the way how the electrical signal is generated by its temperature change, the way how the signal is transmitted and processed, and the appropriate materials used. In fact, some progress has been made in the above researches, which are briefly introduced in this paper.

2. Physical structure and working principle

When creating temperature-sensitive conductive Composites, conductive fillers travel through a soft polymer matrix to create a Complicated conducting net. The conductivity of this network is greatly influenced by the load level and uniform dispersion of the conductive fillings. The total Resistivity reduces gradually as filler content increases until it reaches the Seepage theory loading threshold, when it suddenly drops by many orders of Magnitude and then stabilizes. The electrical behavior of the conductive composite Material is mostly determined by the microstructure of the conductive network as temperature changes. The resistivity of the conducting compound Changes significantly with temperature because the microstructure of the Conductive network is altered by the difference in the coefficient of thermal Expansion of the matrix and filling. The distance between the two Adjacent fillers grows when the temperature rises as a result of the polymer Matrix's volume expansion, which raises resistance. The resistance of the Composite in this temperature range can alter by orders of magnitude as the Temperature rises, up until the polymer volume expansion hits the maximum melting point [2].

The reason why the resistance of composite Conductive materials changes has numerous causes. One example is that, as shown in figure 1, the CNFs (Carbon Nanofibers) that have been manufactured are mainly composed of disordered layered Graphite structure. While the lone electrons not involved in hybridization Overlap each other to form a delocalized bond, each carbon atom hybridizes with Three adjacent carbon atoms in sp^2 mode to form a covalent bond. In the plane of in a carbon atom, those delocalized electrons can go around freely. Some of the Atom's previously bound electrons gain energy as the temperature increases, becoming free electrons that can move from the valence band to the conduction Band. The basic principle of temperature measurement in the sensing model is that the CNFs become more concentrated and mobile, Reduction of resistance [3].

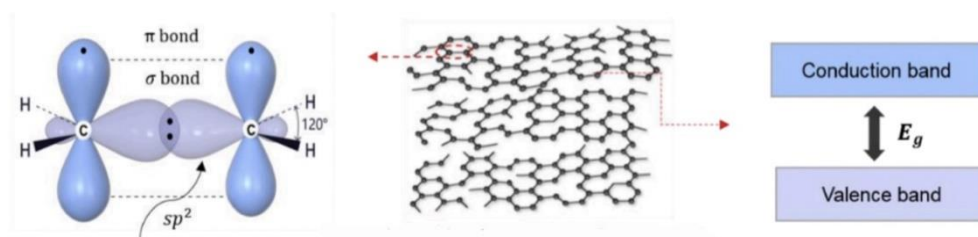


Figure 1. The structure of the layered graphite [3].

Several theories of conductive mechanisms have been proposed to reveal the thermo-mechanical and electromechanical effects of thermosensitive conductive composites, such as percolation, conduction pathway, hopping transport mechanism, tunnel effect and electric field emission theories.

What is discussed above can be regarded as the working principle of the thermal resistance temperature sensor. As a matter of fact, in addition to thermal resistance temperature sensors, common temperature sensors also include thermocouple. Fig 2 illustrates the basic mode of thermocouple temperature sensor.

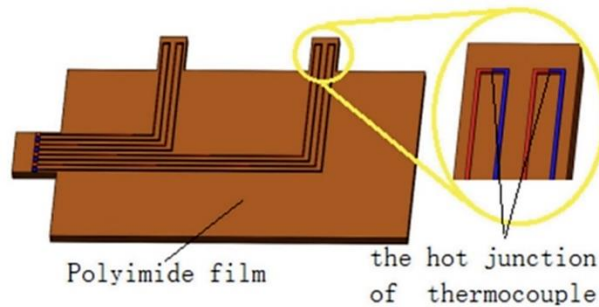


Figure 2. Physical mode of the flexible thermocouple file sensor [4].

Briefly, the working principle of thermocouple is based on a combination of the Seebeck Effect and Heat Transfer Rule. Two different metal wires connected to two Nodes form a thermocouple. One of the nodes acts as a thermal junction, and the Temperature around it changes as the external environment changes. The free It acts as a cold junction and the temperature around it remains constant. Therefore, the temperature difference between the hot and cold junctions leads to create a heat potential. To explain this theory However, it may be assumed that the cold end and the hot end carrier Concentration are the same at the beginning, and with the change of Temperature, the average motion speed of the hot end carrier increases, the Energy increases, so does the chemical potential. As a result, carriers Move toward the cold end and accumulate, and these carriers create an electric Field that prevents other carriers from continuing to move toward the cold end, and eventually the two reach equilibrium, with the potential difference between The cold and hot ends called the Seebeck voltage. Here's a simple explanation For the Seebeck effect.

Thermocouple temperature sensors have some disadvantages in terms of accuracy and stability of temperature measurement. The “non-uniform potential” of thermocouple will directly affect the temperature result. The homogeneity of a thermocouple refers to the degree of homogeneity of the pole material of a thermocouple. Suppose the material of the two thermal electrodes is not uniform, and the thermal electrode is in a temperature gradient field. In that case, the thermocouple will produce an attached heating potential, that is, the “non-uniform potential”, which will affect the total thermoelectric potential of the thermocouple circuit. Therefore, the existence of “non-uniform potential” will cause the thermoelectric characteristics of the thermocouple to change, thus reducing the temperature measurement accuracy. The contamination, oxidation reaction, reduction reaction, high temperature reaction and embrittlement reaction of the thermocouple thermal electrode will cause the measurement error of thermocouple temperature sensor [5].

Based on the above demonstration, it is generally believed that thermal resistance temperature sensors are more suitable.

Since the temperature of the human body is continuously changing, the resistance value of the thermistor will also change, if there is a voltage source in the circuit, the current generated will change with the resistance, the change of the electrical signal can reflect the temperature change, which is the basic principle of the thermal resistance temperature sensor can detect the temperature.

In order to allow users to easily understand the temperature situation, the man-machine display interface can be designed, which is based on the Bluetooth module, through which the current signal of the temperature sensor can be sorted out and transmitted to the hardware of the remote terminal. In fact, the Bluetooth module also includes ADC(Analog-to-Digital Converter), which can convert the Analog signal collected into a Digital signal for easy transmission. Fig 3 shows the circuit structure of this temperature sensor.

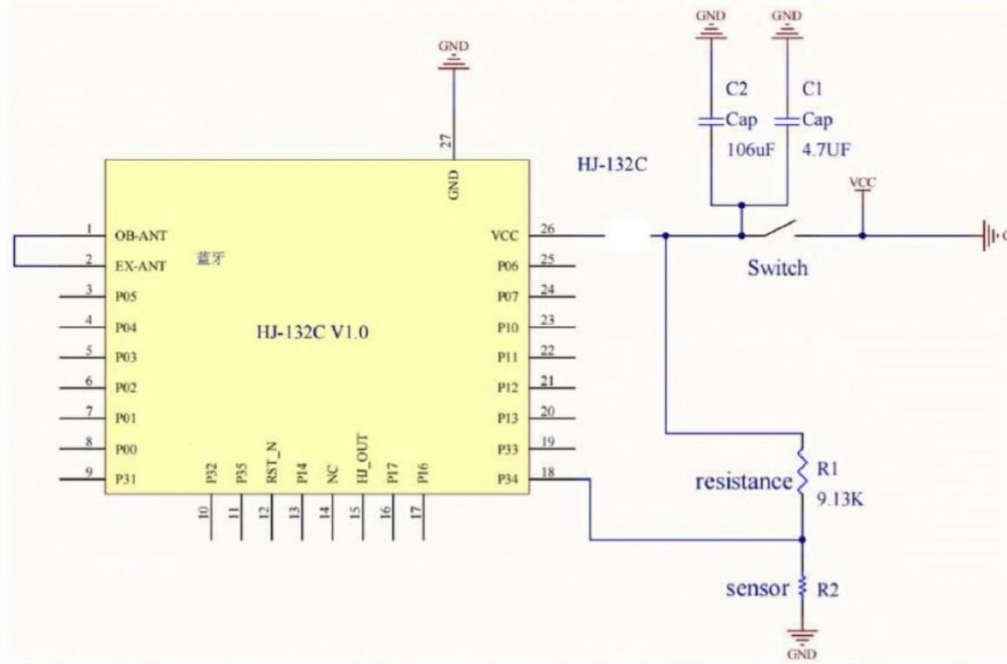


Figure 3. The circuit principle design of the wearable flexible temperature sensor [6].

The data of the wearable flexible temperature sensor system is initially generated by the flexible temperature sensor sensing the body temperature information, and finally displayed in the display interface, and the data is transmitted level by level. To summarize this roughly, it is roughly after three data transfers. The first data transfer is the ADC module on the Bluetooth module to collect the information of the flexible temperature sensor. The second data transfer is that the Bluetooth module transmits information from the flexible temperature sensor to the terminal hardware. The third data transmission is that the terminal hardware transmits information to the terminal software, that is, the display interface of the upper computer.

As mentioned above, the human-machine display interface receives the electrical signal transmitted by the terminal hardware, even if it is converted into a change in the sensor thermistor resistance value through the background, but this is still not the change in human body temperature that we need to measure, so the human-machine display interface needs to be told what kind of resistance change corresponds to what kind of temperature value, then we need to provide a benchmark for the human-machine display interface. It is convenient for it to determine the relationship between the change of resistance value and the change of temperature. The solution is to conduct many experiments, use the data obtained from these experiments to fit a functional curve, and use this fitted curve as the above reference.

Due to the inevitable data loss and error in data transmission, there will always be some accidental data in the fitting process, and these data fluctuate greatly, which will affect the accuracy and credibility of the fitting. To deal with this problem, filtering algorithms filter through these random data [6].

3. Material

The material for a wearable temperature sensor based on electronic skin is also important. This sensor is directly worn on the body, for reasons such as portability, traditional bulky batteries and some materials will no longer be suitable.

Traditional electronics typically use traditional materials such as silicon and metal, which have a surface elastic modulus of 140 to 600kPa, and are so bulky that they cannot be widely used in wearable devices that require large deformation. The excellent ductility of the material allows for high temporal and spatial resolution of the contact between the device and the skin with its complex dynamic structure,

making the measurement more accurate and also enhancing the signal collected from the skin interface [7].

Polymers are the most promising intrinsically stretchable materials for substrate materials because of their superior elasticity and stability, such as Polydimethylsiloxane (PDMS), PU (PU) and Ecoflex (EP). Azide cross-linking chemistry is used for semiconductor polymers to make intrinsically stretchable semiconductor polymer. The energy caused by strain can be dissipated by reversible breaking and recombining of weak hydrogen bond and electrostatic interactions in the polymer. To further improve the tensile properties of the dynamic non-covalent crosslinks of flexible polymeric chains can be added. Plasticizers and nanoconfinement effects have been shown to improve the inherent ductility of conjugated polymers and can be used to design stretchability.

Specifically, the selection of the flexible substrate in the wearable flexible temperature sensor needs to meet the following points: 1. Raw object compatibility, wearable flexible temperature sensors are mostly in direct contact with the human skin surface, so the selection of flexible substrates needs to be harmless to the human body. 2. With good flexibility and extensibility, wearable flexible temperature sensors can continuously measure human surface temperature at multiple points to better communicate with human skin [8].

Surface contact, flexible substrate needs to have a certain degree of flexibility and extensibility. 3. Manufacture of flexible devices Manufacturing involves different process steps and requires high temperatures above at least 300 ° C, therefore, excellent heat resistance performance is the key requirement of flexible substrate. The flexible substrate should also have a traditional thermistor sensor base Some characteristics of the plate include insulation, hydrophobicity, etc.

A common and practical method is as follows: The pre-cleaned Capton substrate supports the device. Horizontally, the ZnO nanowire array is the core material of the device. Manufacturing processes (deposition of digital interelectrode patterns), including lithography and electron beam evaporation processes. ZnO nanowires (length :12 μm) can pass through the electrode pair (distance :5 μm). The distance between adjacent electrode pairs is 20 μm, ensuring that all ZnO nanowires' C-axis direction is the same [9].

The above is a common process for making flexible electronic skin, which enables the electronic skin to have the characteristics of low mass and breathability, while not affecting its function.

4. Specific research work introduction

Due to the increasing demand for highly sensitive and flexible temperature sensor systems. A fully flexible NiO temperature sensor system was previously investigated using a single-step, monolithic laser reduction sintering (m-LRS). Figure 4 shows the resistance of three Ni Electrodes in the 25-70 ° C temperature range. The antenna Ni electrode produced by m-LRS method has the same thermal resistance ratio (TCR) in comparison to standard Ni. The TCR properties of Ni-NiO-Ni structure is remarkably high as $-9.2\% \text{ }^{\circ}\text{C}^{-1}$. Over the entire measurement range (25-70 ° C), the material constant of the resulting thermistor is calculated to be 7350 K. In particular, very high values of 8162 K are obtained at ambient temperature. Their results are believed to be the highest sensitivity ever reported for a thermistor [10].

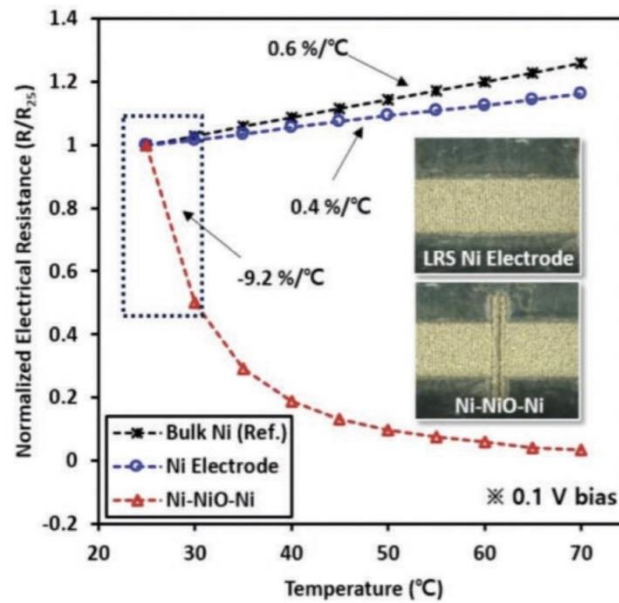


Figure 4. Test results of electrodes of different materials [10].

At the same time, a good wearable temperature sensor needs to be able to accurately and sensitively reflect temperature changes, but also needs to be able to quickly respond to temperature changes, but also needs to be able to remain stable after multiple measurements.

In order to achieve the above functions, researchers have conducted a number of tests on the wearable temperature sensor based on electronic skin prepared by it. To test its linearity and stability, they heated the sensor gradiently. The results show that in the measuring range of 20~100 °C, the experimental data points basically coincide with the fitting line, the linearity error is 1.5%, and the sensitivity is $3.2 \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$; To test its response time, they measured the temperature gradient signal on the sensor by adding drops of water. The result is that the nickel film is only separated from the water drop by a 25 μm thick PI film, so the response time is only 1.5 s. After the droplet is gradually cooled to room temperature by air, the relative change in resistance is also reduced to 1.5×10^{-2} ; To test its cyclic stability, they cycled the sensor repeatedly on a hot table. The result shows that the resistance varies from 623.2 to 717.8 Ω at the initial stage. After cyclic temperature loading, the resistance value corresponding to 40 °C increases by 0.85 Ω and the drift rate is 0.14%. Finally, they got a wearable temperature sensor that can better meet the above three requirements [11].

Another important factor to measure the performance of a wearable temperature sensor is its ability to resist external interference, and strain is a common external interference, which can not be ignored. For this reason, researchers designed and tested a sensor, fixed on a ring with different curvatures (corresponding to different degrees of strain). At 20~50°C, the designed flexible temperature sensor is installed on a ring with a curvature of 0~0.5. The bending degree of the flexible temperature sensor can be precisely controlled by rings with different curvatures, and then the strain can be quantitatively controlled. The output resistance values of 5 groups of sensors were compared with the output resistance of horizontally placed flexible temperature sensors to verify the ability of sensors to resist strain interference. The average value of each group of sensors recorded 100 times of data showed that the flexible temperature sensor displayed consistent signal output on the surface of rings with different curvatures. The result is that the biosensor they designed has better resistance to strain interference than traditional flexible temperature sensors [8].

The above is the design and research of some researchers on wearable temperature sensors, which has reference significance.

5. Practical Application

Electronic skin based biosensors can have many functions and can detect temperature, humidity, gas, strain, etc.

This wearable temperature sensor based on electronic skin has great potential in building better human-machine interaction, to exemplify, for intelligent humanoid robots, when they perform tasks such as picking up a glass of water, or performing high-risk tasks in place of humans, they need to have the same temperature perception as humans so that they can react to the temperature in a timely manner to avoid being damaged by extreme heat or cold.

In addition, when building an artificial limb, we need to consider its ability to sense temperature, and it needs to be able to accurately detect temperature, so that the disabled person using it can perform like a normal person.

For wearable temperature sensors, their function is even more important. Accurately detecting local temperature variations in real time in biological tissues, without considering large deformations, is essential for understanding the thermal principles of homeostasis, assessing complex health conditions, and further opening up possibilities for building smart healthcare and health systems [2].

At the same time, as an important indicator of human health, body temperature carries psychological information of health and can reflect the health status of the human body, such as physiological movement, emotion, and metabolism. It can also be used to determine wound healing, wound inflammation status, and for the early diagnosis of cardiovascular and pulmonary diseases. Taking the metabolic condition of body temperature response as an example, if the body temperature drops by 1 Celsius, activates a core of the body, causing primary metabolism to fall by six percent. 7%. The body's metabolism is accelerated as the outside temperature falls. The production of more hormones (for example, thyroid, adrenal, and so on), allowing the body to generate more energy. When the outside temperature rises, the opposite is true. The body adjusts its metabolism to maintain a normal body temperature. Therefore, body temperature can reflect whether the metabolism of the body is healthy, and this can assist in assessing physical health [12].

A variety of wearable temperature sensors have been developed at present, However, since humans wear this kind of sensor and it will touch people's skin directly, a lot of questions cannot be neglected, Such as some sensors are too heavy, some sensors will always attract people's attention to it, in addition, after wearing this sensor for a long time, people can feel itchy and humid heat because of air permeability problems. Because the materials used to make these temperature sensors are lightweight, breathable, and have excellent sensing capabilities, they can meet all these situations' needs, whether for humanoid, robotic and artificial limbs, or directly for real People. In short, the Wearable Temperature Sensor Based on Flexible Electronic Skin can precisely detect temperature. With excellent sensing ability, it can realize multiple functions such as environmental temperature detection and Expiration Temperature Monitoring Electronic skin can simulate the multi-dimensional information and perception function of human skin, and has been widely concerned in human-computer interaction, intelligent prosthetics, Intelligent robots, health monitoring, medical diagnosis and other aspects, so it has a wide range of uses [3].

6. Conclusion

Wearable temperature sensors based on electronic skin play an important role in the realization of human-computer interaction, engineering perception and humanoid perception due to their powerful functions. Since body temperature is an important physiological index of the human body, accurate measurement of body temperature is the main function of this kind of biosensor. In order to improve its performance and usability as much as possible, researchers have carried out many experiments, their purpose is to make their designed sensor can accurately and linearly measure temperature, but also to be able to respond quickly to temperature changes, while to ensure its durability but also improve its cycle stability, in order to adapt to different environments, but also to ensure that its measurement accuracy is not affected by external conditions. In addition, in order to make users more comfortable, they also need to explore lightweight, flexible and malleable materials, while not harmful

to the human body and can ensure the full realization of its function. In order to achieve the above goals, they went deep into the principle level, understood the reasons why temperature causes changes in electrical signals, and analyzed the advantages and disadvantages of thermal resistance and thermocouple temperature sensors. For the electrical signal, they have taken a suitable way to collect, transmit and filter the data so that it can accurately reflect the temperature change, and for this, they have designed elaborate circuits. For the choice of materials, they start from the micro scale, using nano-components to manufacture flexible substrates, making their sensors portable, lightweight, flexible and malleable, and fully functional. In order to achieve the desired goal, they conducted many experiments on the designed biosensor. In the future, researchers should be committed to exploring more advanced materials, which will greatly improve the water resistance, air permeability, and ductility, in addition, more sophisticated circuit structures and components will make temperature measurement more accurate, and measurement errors caused by external conditions will be further reduced.

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Regulatory effects and clinical application of panax-based compound formula on chronic diseases

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Abstract. Hypertension, diabetes, and hyperlipidemia are common chronic diseases today and can lead to severe complications. Panax-based compound formula, as a comprehensive traditional Chinese medicine formulation, incorporates various components such as Panax, exerting multiple pharmacological effects, including nourishing the kidney qi, strengthening the heart, vasodilation, and increasing blood flow. Through the study of multiple cases, including patients with hypotension, heart disease, diabetes, hyperlipidemia, as well as early and long-term hypertension, preliminary evidence has been provided, indicating the potential positive effects of this compound in different types of hypertensive and diabetic patients. It demonstrates clear antiarrhythmic effects and effectively reduces blood pressure, improves heart failure, and alleviates hyperlipidemia. However, further research and clinical trials are required to verify its long-term therapeutic effects and safety. It is crucial to develop personalized treatment plans when using this compound formula.

Keywords: Chronic diseases, Panax-based compound formula, Pharmacological effects, Case studies

1. Introduction

Hypertension is widely considered a primary risk factor for cardiovascular diseases. It can be categorized into different types based on its etiology and mechanism, including renal hypertension and cervical hypertension. Renal hypertension is commonly caused by renal dysfunction, leading to signals in the heart that require increased pumping force to compensate for insufficient renal blood supply. On the other hand, cervical hypertension is often associated with vascular constriction due to skeletal misalignment, requiring the heart to exert more force to deliver blood throughout the body. Excessive reabsorption of sugar by the kidneys is also one of the causative mechanisms of diabetes. Additionally, the coexistence of hypertension and hyperlipidemia not only affects the cardiovascular system's health but may also give rise to various severe complications such as stroke, coronary heart disease, and heart failure.

According to the World Health Organization, the prevalence of hypertension, hyperlipidemia, and diabetes is continuously increasing globally, posing a significant threat to public health and individual well-being. Traditional approaches for treating chronic diseases primarily involve drug therapy and lifestyle interventions, such as dietary modifications, exercise, and weight control. However, these

methods only provide symptomatic relief and do not fundamentally address the kidney yin deficiency in renal hypertension.

Panax, a traditional Chinese medicine with a long history, has long been believed to have tonifying effects, especially on kidney function. Rich in components such as dammarane-type saponins, ginsenoside, and panaxatriol, Panax is described in traditional herbal texts as a medicine for “tonifying qi and nourishing blood, assisting in essence nourishment, and spirit cultivation.” In clinical practice, Panax has been proven to have remarkable effects in tonifying bone marrow and kidney qi, mitigating the co-action of the liver in hyperlipidemia, and relieving kidney yin deficiency. Furthermore, Panax can increase blood saturation in the heart [1], enhance cardiac contractility, dilate blood vessels, increase blood flow, and thus regulate blood pressure and blood sugar. Compared to traditional deer antler, the reinforcing and bone marrow-tonifying effects of Panax are more significant, especially for the elderly. Deer antler may be too yang-invigorating, leading to spermatorrhea in the elderly. On the other hand, Panax performs exceptionally well in urgent cases of rescuing collapsed yang, as its efficacy has been confirmed through centuries of traditional Chinese medical practice.

Through this study, we aim to provide a new option for the comprehensive treatment of chronic diseases such as hypertension, hyperlipidemia, and diabetes. This option not only focuses on symptomatic treatment but also emphasizes the restoration of kidney function and the enhancement of spinal cord blood production capacity, thereby combating the root causes of chronic diseases such as hypertension, hyperlipidemia, and diabetes. We believe that this study will provide strong support for improving the quality of life of chronic disease patients and reducing the risk of related complications.

2. Current Treatment Status of Chronic Diseases

2.1. Renal Hypertension

Based on different causes and mechanisms, renal hypertension can be further divided into two main types: volume-dependent and renin-dependent.

2.1.1. Volume-dependent Hypertension. In volume-dependent hypertension, the kidney’s processing capacity is impaired, leading to the ineffective elimination of excess sodium and water by the kidneys. When the intake of sodium and water by the body exceeds the kidneys’ excretory capacity, water-sodium retention occurs. This retention can primarily occur within the blood vessels, leading to an expansion of blood volume and thus causing hypertension. Simultaneously, water-sodium retention can also increase the water-sodium content within the smooth muscle cells of the blood vessels, making the vessel walls thicker, less elastic, and increasing vascular resistance, thereby reducing the responsiveness of the vessels to catecholamines. Additionally, water-sodium retention can increase the affinity of angiotensin II for vascular receptors, leading to elevated blood pressure even if the angiotensin levels are normal.

2.1.2. Renin-dependent Hypertension. The pathogenesis of renin-dependent hypertension usually involves factors such as renal artery stenosis, decreased renal perfusion pressure, renal parenchymal disease, and secretion of renin by cell tumors. These conditions can cause the juxtaglomerular cells in the kidneys to release a large amount of renin, leading to increased activity of angiotensin II, which in turn causes spasm and hardening of the small arteries throughout the body, resulting in hypertension. Additionally, renin and angiotensin II can also stimulate increased aldosterone secretion, further causing sodium and water retention, increasing blood volume, and consequently leading to hypertension. Renal parenchymal hypertension can be caused by various kidney diseases, and the chances of developing hypertension vary with different types of kidney diseases. Therefore, the clinical manifestations of renal hypertension are diverse.

2.2. Diabetes

Diabetes is caused by defects in insulin secretion, its action, or both, leading to chronic damage and dysfunction in various tissues, especially the eyes, kidneys, heart, blood vessels, and nerves. If the

patient already has primary renal glycosuria or secondary renal glycosuria, it is easy to become a precursor to diabetes. Additionally, organic kidney damage patients such as nephritis and multiple myeloma are prone to renal glycosuria. Some secondary renal glycosuria is often accompanied by other renal tubular dysfunctions and typical clinical manifestations of the primary disease.

2.3. Hyperlipidemia

Hyperlipidemia in some patients is primary, related to congenital and genetic factors, while secondary hyperlipidemia mostly occurs in metabolic disorders such as diabetes and hypertension, as well as liver and kidney diseases or kidney deficiency. Environmental factors such as dietary and lifestyle habits can also lead to hyperlipidemia. Hyperlipidemia refers to excessively high blood lipid levels, which can directly cause severe diseases harmful to human health, such as atherosclerosis, coronary heart disease, and pancreatitis.

2.4. Current Treatment Status

2.4.1. Pharmacological Treatment. Pharmacological treatment is a common method in the management of chronic diseases, primarily focused on control. Various antihypertensive drugs such as diuretics, β -blockers, calcium channel blockers, ACE inhibitors, ARBs, as well as oral sulfonylureas, biguanides for hypoglycemia, α -glucosidase inhibitors, insulin sensitizers, glinides, or insulin injections, are widely used for the treatment of hypertension and diabetes. These medications have varying mechanisms but all work through different pathways to control the condition, such as vasodilation, reducing cardiac contractility, promoting urine excretion, balancing hormone levels within the body, and alleviating the burden on the pancreas. However, pharmacological treatment typically requires long-term maintenance and may be accompanied by some side effects.

2.4.2. Lifestyle Intervention. In addition to pharmacological treatment, lifestyle modification is also an important component of chronic disease management. Lifestyle interventions include dietary improvements, increased physical activity, weight reduction, smoking cessation, and alcohol intake restriction. These measures help in reducing weight, improving cardiovascular health, lowering blood pressure and lipid levels, controlling blood sugar, and reducing the risk of related complications. Lifestyle interventions also aid in improving the quality of life for patients and can reduce dependence on pharmacological treatments.

3. Ingredients and Preparation of Compound of Panax Ginseng Medicinal and Edible Origin

3.1. Main Ingredients

The compound of Panax ginseng medicinal and edible origin is primarily composed of Panax ginseng as the main ingredient, combined with various botanical medicinal ingredients. The main ingredients are as follows:

Panax ginseng: This is the core component of the compound, renowned for its remarkable effects in replenishing qi, nourishing yin, and aiding in the enhancement of vitality. Panax ginseng contains abundant components such as ginsenosides, dammarane saponins, panaxatriol saponins, and panaxadiol saponins. These components help in enhancing kidney qi, alleviating kidney yin deficiency, increasing blood saturation in the heart, strengthening cardiac function, dilating blood vessels, increasing blood flow, and thereby playing a positive role in regulating blood pressure.

Lycium barbarum (Goji berries): It possesses properties that nourish the liver and kidneys, replenish blood and nourish yin, and nourish yin and yang. It balances yin and yang, promotes the flow of energy within the body, and has a positive effect on liver and kidney function, synergizing with Panax ginseng to enhance the production of vital energy.

Morus fruit (Mulberries): It has the effect of nourishing the liver and kidney, nourishing yin, and promoting blood. It aids in nourishing the kidneys and nourishing yin, working in synergy with Panax ginseng to strengthen kidney function.

Polygonatum sibiricum (King Solomon's seal): It has the functions of nourishing yin and replenishing the kidney, generating fluids, and moistening the lungs. It assists in the restoration of kidney function while improving lung function, enhancing overall immunity.

Black sesame: Rich in high-quality fats, proteins, and trace elements. It aids in the absorption of the effective components of Panax ginseng, providing necessary fats and proteins, and supplying the required nutrients for the kidneys and heart.

Chinese black dates: They help in nourishing the blood, nourishing the heart, invigorating the spleen, and calming the mind. They play a balancing and harmonizing role in the combination, helping to alleviate anxiety and tension, while strengthening cardiac function.

Walnuts: They contain abundant unsaturated fatty acids, vitamin E, and trace elements. They benefit heart health, enhance cardiac function, and provide essential fatty acids for chemical reactions within the body.

Sour jujube seeds: They have calming and heart-nourishing effects, aiding in calming emotions, reducing anxiety, and improving sleep.

Mint: Mint disperses wind, clears heat, and detoxifies, aiding in digestion. Its role in this compound is to dispel internal heat, ensuring that the consumption of Panax ginseng does not lead to symptoms of excessive internal heat, while also assisting in digestion.

3.2. Preparation Method

The key steps in preparing the compound of Panax ginseng medicinal and edible origin include herbal extraction, mixing, and forming.

3.2.1. *Herbal Extraction.* Firstly, various herbs undergo meticulous processing to ensure their purity and the preservation of active components. Water extraction technology is employed to thoroughly mix the herbs with water for extraction. This technique aids in extracting the effective components of the herbs, preparing high-purity traditional Chinese medicine extracts.

3.2.2. *Mixing.* The extracted traditional Chinese medicine extracts are mixed together to ensure the synergistic effects of various herbs. The proportions of various herbs are determined based on clinical needs during mixing to achieve the best therapeutic effects. Additionally, honey is added to the mixture to enhance the convenience of medication administration.

3.2.3. *Forming.* Finally, the mixture is formed into pills or other easily consumable forms for patients. This ensures that patients receive accurate dosages and facilitates ease of administration.

3.3. Traditional Chinese Medicine Extraction Technology

To better preserve and enhance the active components of traditional Chinese medicine, water extraction technology is adopted. This technology aids in extracting high-purity active components from the herbs to ensure the efficacy of traditional Chinese medicine. Water extraction is a relatively gentle extraction method that can reduce losses and damage to herbal ingredients. Moreover, the use of high-purity traditional Chinese medicine extracts allows for better absorption by the human body, enhancing the medicinal effects.

During water extraction, the herbs are first ground and crushed to increase their surface area, then mixed with an appropriate amount of water. The mixture is then heated, usually at a moderate temperature, to aid in the release of active components. During the extraction process, the active components in the herbs are transferred to the water, forming a high concentration of extract. This extract undergoes filtration and concentration to remove impurities and moisture, ultimately resulting in high-purity traditional Chinese medicine extracts. Water extraction is a relatively gentle method that helps

preserve the active components of the herbs, making them more effective. Through water extraction, the obtained traditional Chinese medicine extracts generally have higher purity, reducing the presence of impurities and aiding the effectiveness of the medicine. Water extraction technology helps reduce losses and damage to herbal ingredients, fully utilizing the potential of the herbs.

4. Pharmacological Effects of the Compound of Panax Ginseng Medicinal and Edible Origin

The compound of Panax ginseng medicinal and edible origin is a comprehensive traditional Chinese medicine preparation containing various herbal components, primarily centered around Panax ginseng. This compound exhibits multiple pharmacological effects, including bone marrow nourishment, kidney qi replenishment, cardiac stimulation, vasodilation, and increased blood flow. The following sections provide a detailed elucidation of its pharmacological effects.

4.1. Kidney Qi Replenishment

Panax ginseng, a well-known herbal remedy in traditional Chinese medicine, is widely utilized for its kidney nourishing properties, believed to aid in improving kidney function. In the context of traditional Chinese medicine theory, the kidneys are regarded as the foundation of one's innate constitution and the fundamental source of life. Kidney deficiency is a common pathological condition in traditional Chinese medicine, typically manifesting as fatigue, soreness and weakness of the lower back and knees, aversion to cold, dizziness, among other symptoms. The kidney-nourishing effect of Panax ginseng helps ameliorate these symptoms and enhances the body's vitality. Its function in enhancing renal metabolism contributes to its efficacy in addressing chronic conditions such as kidney stone formation and uric acid-related gout.

4.2. Cardiac Stimulation

Panax ginseng exhibits a positive inotropic effect, enhancing the heart's contractile force and pumping efficiency, thereby increasing the blood flow to the heart and kidneys. This is particularly crucial for patients with hypertension, as the heart requires greater force to propel blood to meet the body's demands. The cardiac stimulatory effect of Panax ginseng can alleviate the burden on the heart [2] and enhance its function. The active components of Panax ginseng possess the ability to scavenge free radicals. MDA, a lipid peroxide formed during myocardial ischemia, is the most important metabolite reflecting tissue lipid peroxidation in the body. Ligation of the coronary artery leads to a gradual increase in blood MDA levels, while SOD can lower these levels, indicating the involvement of oxygen free radicals in myocardial ischemic injury, and SOD can mitigate myocardial ischemic injury. Ginsenosides can increase SOD activity [3], directly quenching O_2 , and eliminating H_2O_2 -induced damage to the body.

Existing research has also demonstrated through animal experiments that total ginsenoside has a protective effect against arrhythmias [4], significantly inhibiting QRS prolongation, increasing T-wave amplitude, and reducing the incidence of ventricular arrhythmias.

4.3. Vasodilation and Softening

Existing research has demonstrated that both panaxadiol and panaxatriol saponins can significantly accelerate the blood flow velocity in arterioles and venules [5], increase the diameter, number, and openness of microvessels, improve blood flow dynamics, and inhibit the formation of blood clots. The components of the compound of Panax ginseng medicinal and edible origin possess vasodilatory effects, helping to dilate blood vessels and reduce vascular resistance. This aids in lowering blood pressure, as blood can flow more easily through dilated vessels, reducing the workload on the heart. Vasodilation also contributes to improved blood supply to various parts of the body, helping to enhance microcirculation and reduce tissue ischemia.

4.4. Bone Marrow Nourishment and Increased Blood Flow

The components of the compound of Panax ginseng medicinal and edible origin can increase bone marrow content, improve blood flow, enhance cardiac output, and stimulate bone marrow hematopoiesis.

This helps improve blood supply to various tissues and organs, reduces the risk of Parkinson's disease, stimulates lumbar nerve repair, and alleviates ankylosing spondylitis. Moreover, it effectively alleviates tissue ischemic symptoms such as dizziness, vertigo, and fatigue that may occur in patients with hypertension and hyperlipidemia. Increased blood flow also helps eliminate excess waste and toxins from the body, promoting overall metabolism.

4.5. Lipid-lowering Effects

A substantial body of research has indicated that ginseng protein has a lipid-lowering effect on animal models of hyperlipidemia. These research findings suggest that ginseng protein can significantly reduce serum total cholesterol (TC) and triglyceride (TG) levels in hyperlipidemia models. It can also increase the content of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver and reduce alkaline phosphatase (ALP) levels [6].

4.6. Blood Glucose Reduction

Research has confirmed that oligopeptide components in ginseng have a potential inhibitory effect on α -glucosidase [7]. Ginseng demonstrates pharmacological effects such as blood glucose reduction, immune regulation, anti-tumor activity, cardiovascular regulation, antioxidant properties, and anti-aging effects. Experimental evidence has shown that ginseng extracts can lower blood glucose levels, reduce ketones, and promote glucose absorption in diabetes. It has been verified that the water extract of ginseng can not only lower blood glucose levels in mice and improve their glucose tolerance, but also reduce serum triglycerides and increase insulin sensitivity [8].

4.7. Clinical Application Prospects

The compound of Panax ginseng medicinal and edible origin exhibits multiple pharmacological effects, particularly suitable for patients with renal hypertension. Renal hypertension is commonly associated with renal dysfunction, and the kidney-nourishing effect of Panax ginseng can specifically improve kidney function. Additionally, its pharmacological effects of cardiac stimulation, vasodilation, and increased blood flow can synergistically help regulate blood pressure and reduce the risk of hypertension. There is also a significant body of experimental and clinical research on its effectiveness in hyperlipidemia and diabetes. As the majority of patients are elderly, the kidney and bone marrow nourishing effects of the compound of Panax ginseng medicinal and edible origin have helped alleviate the suffering of some elderly patients from lower back and leg pain. The comprehensive effects are encouraging.

With the increasing understanding and acceptance of traditional Chinese medicine, the compound of Panax ginseng medicinal and edible origin has a promising application outlook in the treatment of hypertension. It can serve not only as an alternative option to traditional treatments but also be combined with conventional antihypertensive drugs to enhance treatment efficacy. Moreover, the compound has good safety and tolerability, reducing the risk of adverse reactions in patients.

In summary, the compound of Panax ginseng medicinal and edible origin, with its multiple pharmacological effects, provides a comprehensive solution for the treatment of renal hypertension, diabetes, and hyperlipidemia. It has a broad clinical application prospect, offering the potential to provide more effective treatment options for patients with chronic diseases and improve their quality of life. However, despite its many advantages, further research and clinical trials are needed to verify its long-term efficacy and safety.

5. Clinical Research and Case Studies

5.1. Case Study One: Treatment Experience of Ms. Li

Ms. Li, a female aged over 60, had been suffering from hypotension for an extended period. She took the compound of Panax ginseng medicinal and edible origin in pill form for three months, with a daily dosage of 2 pills. As the treatment progressed, her blood pressure gradually returned to normal levels.

This case provides clear evidence of the compound's effectiveness. Ms. Li's treatment experience demonstrates that the compound can significantly elevate the blood pressure levels of hypotensive patients without any significant adverse reactions during the course of treatment.

5.2. Case Study Two: Treatment Experience of Ms. Wang

Ms. Wang, a female aged between 30 and 40, experienced postpartum cardiac discomfort and was suspected of having coronary heart disease after hospital examination, along with a diagnosis of depression and anxiety. She used the compound of Panax ginseng medicinal and edible origin in pill form for one month, taking 2 pills daily. As the treatment progressed, her symptoms of palpitations and dyspnea were significantly alleviated. This case indicates that the compound can improve cardiovascular disease symptoms and also has a positive effect on emotional disorders.

5.3. Case Study Three: Treatment Experience of Mr. Zeng

Mr. Zeng, a male aged over 60, exhibited early symptoms of hypertension along with high blood lipids and had not received any antihypertensive drug treatment. He took the compound of Panax ginseng medicinal and edible origin in pill form for three months, with a daily dosage of 2 pills, resulting in a gradual return of both blood pressure and blood lipid levels to normal. This case reveals the effectiveness of the compound in early-stage hypertensive patients. Mr. Zeng's treatment experience demonstrates that the compound can play a significant role in the early stages of hypertension without the need for traditional antihypertensive drugs.

5.4. Case Study Four: Treatment Experience of Mr. Yu

Mr. Yu, a male aged over 60, suffered from hypertension accompanied by hyperlipidemia, kidney stones, and had undergone coronary bypass surgery several years ago. He took the compound of Panax ginseng medicinal and edible origin in pill form for six months, with a daily dosage of 4 pills. His blood pressure returned to normal levels, lipid indicators decreased, and there was good softening of the blood vessels, with a corresponding reduction in kidney stones. This case demonstrates the significant effects of long-term use of the compound in controlling blood pressure in patients with complex diseases. Mr. Yu's treatment experience emphasizes the importance of the compound in the long-term management of patients with complex diseases accompanied by hypertension.

5.5. Case Study Five: Treatment Experience of Ms. Zhang

Ms. Zhang, a female aged over 60, suffered from diabetes. She took the compound of Panax ginseng medicinal and edible origin in pill form for six months, with a daily dosage of 4 pills, resulting in a reduction in blood sugar. This case indicates that long-term use of the compound has significant effects on blood sugar control. Ms. Zhang's treatment experience underscores the importance of the compound in the long-term management of diabetes.

5.6. Case Study Six: Treatment Experience of Ms. Zhuo

Ms. Zhuo, a female aged over 70, had diabetes and knee cartilage degeneration, often experiencing knee pain at night, affecting her sleep. She took the compound of Panax ginseng medicinal and edible origin in pill form for six months, with a daily dosage of 4 pills. As a result, her blood sugar decreased, and the pain in her knee bones significantly reduced, leading to an improvement in sleep quality. This case illustrates the significant effects of the long-term use of the compound on blood sugar control and the alleviation of waist and knee pain caused by osteoporosis. Ms. Zhuo's treatment experience emphasizes the importance of the compound in the long-term management of diabetes.

6. Conclusion

Through the study of multiple cases and the analysis of examples, the potential effectiveness and multifunctionality of the compound of Panax ginseng medicinal and edible origin in the treatment of chronic diseases such as hypertension, diabetes, and hyperlipidemia have been established. This

compound, with *Panax ginseng* as its core ingredient, incorporates the pharmacological effects of various traditional Chinese medicinal herbs, including kidney nourishment, cardiac stimulation, vasodilation, and increased blood flow. These cases demonstrate the compound's positive effects on patients with various chronic diseases, including hypotension, heart disease, diabetes, hyperlipidemia, early-stage hypertension, and long-term hypertension. However, it should be noted that although these preliminary results are encouraging, further research and clinical trials are still necessary to validate the compound's long-term therapeutic effects and safety. Treating chronic diseases is a complex task that usually requires comprehensive treatment methods, including medication, lifestyle changes, and more. Therefore, personalized treatment plans are crucial for each patient. In summary, the compound of *Panax ginseng* medicinal and edible origin represents a comprehensive traditional Chinese medicine treatment approach that offers new options for patients with chronic diseases. It has a broad clinical application prospect, with the potential to improve patients' quality of life and reduce the risk of complications related to chronic diseases. However, when using this compound, it is essential to follow the guidance of a physician and develop personalized treatment plans based on the specific conditions and needs of the patients. This will help better leverage its advantages and provide better medical services for patients with chronic diseases.

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The interplay between sleep, neuroplasticity, and health: Insights from a multi-level analysis

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Abstract. All articles *must* contain an abstract. The abstract text should be formatted using 10 point Times or Times New Roman and indented 25 mm from the left margin. Set the pre-paragraph to 0 pounds and the post-break to 22.7 pounds. Starting on the same page as the abstract. The abstract should give readers concise information about the content of the article and indicate the main results obtained and conclusions drawn. The abstract is not part of the text and should be complete in itself; no table numbers, figure numbers, references or displayed mathematical expressions should be included. It should be suitable for direct inclusion in abstracting services and should not normally exceed 200 words in a single paragraph. Since contemporary information-retrieval systems rely heavily on the content of titles and abstracts to identify relevant articles in literature searches, great care should be taken in constructing both. As the issue of sleep deprivation worsens, worries regarding its wider effects on society's health are raised. The detrimental effects of sleep deprivation and lack of sleep on attention, memory, and neuroplasticity have been the subject of several research. The complicated interplay between sleep and neuroplasticity is examined in this research from both a macro and micro level, with a focus on attention and memory. The study also emphasizes how important environmental factors like exercise and food are in affecting sleep patterns and neuroplasticity. These discoveries not only give sleep and neurological research a fresh angle and biological foundation, but they also offer up new options for therapeutic care. Additionally, they offer a theoretical framework based on neurobiology as well as helpful advice for creating public policy.

Keywords: Sleep, Sleep deprivation, Neuroplasticity, Exercise, Diet.

1. Introduction

Sleep has the extraordinary ability to reduce negative effects, restore health, and enhance learning and memory. However, in the modern era, achieving high-quality, timely sleep has become increasingly challenging. According to the World Health Organization, around a third of the global population (27%) suffer from sleep disorders. The prevalence of various sleep disorders is even more pronounced in China, where 38.2 percent of people have them, affecting more than 300 million people - more than the global average.

Numerous studies have shown that sleep deprivation has significant effects on cognitive function and neuroplasticity of the brain. Scientists have clarified that lack of sleep leads to the accumulation of toxic beta-amyloid metabolites in the brain and the phosphorylation of tau protein. These processes adversely

affect central nervous system plasticity, activate glial cells, trigger nervous system inflammation, induce neuronal DNA damage, and ultimately increase the risk of Alzheimer's disease. Sleep, once considered a miracle cure, has become a critical issue in protecting cognitive health.

As the brain functions, it constantly produces metabolic waste. However, it is during sleep that the brain activates its "clearing mode." Lack of sleep leads to a gradual accumulation of metabolic waste in the brain, which leads to progressive damage. In addition, sleep has various effects on our neuroplasticity. Neuroplasticity refers to the ability of the brain to grow, reorganize, and change the structure and function of neural networks.

Our brains remain neuroplastic throughout our lifespan, even in older adults who experience persistent changes due to environmental influences. Notably, experiments on sleep-deprived mice revealed a worrying finding: synaptic proteins no longer follow the expected cyclical pattern. This striking observation strongly suggests that sleep deprivation disrupts the circadian rhythm of synaptic proteins, leading to significant impairment of neuroplasticity.

These interesting findings from previous experiments raise a whole new set of questions. How exactly does sleep affect neuroplasticity? What substances are involved in this complex process? Also, how does sleep regulation intersect with environmental factors? By synthesizing the broader literature and subsequent synthesis of conclusions, we can shed more light on these issues.

These studies and their summary reveal the profound effects of sleep on neural tissue repair and the rebuilding of synaptic connections, providing valuable insights into the underlying processes. These insights not only open up new avenues for clinical treatment, but also provide a solid theoretical foundation for fields such as neurobiology and psychotherapy, providing new perspectives and possibilities for the development of these disciplines.

2. Macro research on neuroplasticity and sleep

2.1. Sleep deprivation reduces concentration

A considerable decline in cognitive performance is linked to ongoing sleep deprivation. There have been several research on how lack of sleep impacts attention, but four key conclusions have emerged. First, lack of sleep causes a general slowing of reactions. Second, lack of sleep makes people more likely to lapse for extended periods of time (>500 ms) and commit mistakes. Third, lack of sleep makes the time-on-task impact in each test bout stronger. Last but not least, PVT findings during prolonged awake point to the existence of coupled circadian and homeostatic sleep drives[1]. Notably, it causes a notable decline in sustained attention, which is defined by a reduced capacity to focus for lengthy periods of time. Furthermore, alert attention, which involves swift responses to stimuli, also experiences a substantial decline, often resulting in an increased error rate in cognitive tasks. It is worth emphasizing that the adverse effects on attention, reaction speed, and error rates become more pronounced with prolonged periods of sleep deprivation, underscoring the cumulative impact of insufficient sleep on cognitive functioning.

Beyond the cognitive realm, sleep deprivation has far-reaching consequences. Research has shown that it not only impairs behavioral attention but also increases the expenditure of physical energy. This heightened energy expenditure can contribute to feelings of fatigue and lethargy, eroding an individual's motivation for both learning and daily life activities.

Moreover, chronic sleep deprivation has been linked to a higher likelihood of experiencing mood disturbances, such as depression. The persistent lack of restorative sleep can lead to a sense of despondency and a loss of enthusiasm for life and learning, posing significant challenges to overall well-being and quality of life.

2.2. Sleep deprivation affects memory

The influence of sleep on memory is multifaceted and exhibits variability across individuals. In general, sleep is an important process of memory consolidation and information integration, and plays a key role

in learning and remembering new knowledge. Empirical research underscores the substantial impact of obtaining sufficient sleep on enhancing memory quality and effectiveness.

Suboptimal sleep patterns can significantly contribute to cognitive impairment, including memory deficits. Typically, these sleep disturbances are associated with irregular dietary habits, disrupted sleep schedules, prolonged wakefulness into late hours, and heightened psychological distress. Sleep, fundamentally, serves as a vital restorative process for the brain, underpinning cognitive functions. Crucially, the process of neuroplasticity, which governs the brain's capacity to adapt and reorganize, hinges upon the robust delivery of cerebral blood flow. This facilitates the elimination of metabolic waste products while also ensuring the supply of oxygen to active neurons. The cyclical sleep-wake transitions are significantly linked to dynamic changes in cerebral blood flow, and these uncontrollable variations may have a significant impact on the subconscious mechanisms that control neuroplasticity while we sleep [2].

Insufficient amounts of sleep can seriously impede the brain's ability to repair itself, potentially leading to energy and oxygen deficiencies in the cerebral area. As a consequence, these weaknesses may show up as distractions, slowed intellectual processing, and a noticeable loss in memory performance. The negative effects of sleep deprivation on brain neural activity are the cause of these deficits. Lack of sleep negatively affects the complex web of neural connections and jeopardizes the smooth transfer of information that exists between neurons. As a result, it causes significant cognitive deficits that particularly disrupt memory function.

Lack of sleep additionally has the potential to impact mood stability, heighten stress and anxiety, and hinder the intricate process of memory development and retention. Insufficient sleep for prolonged periods has been associated with impaired cognitive performance and a higher risk of neurodegenerative illnesses like Alzheimer's. It is important to remember that everyone has different sleep requirements; some people may require less sleep to maintain normal memory function, whilst others may require longer rest several times. Additionally, the quality of sleep has emerged as a significant factor in memory preservation, with continuous deep sleep being essential for memory consolidation.

Therefore, in addition to sleep time, carefully creating a favorable sleep environment and developing healthy sleep habits are crucial to maintaining memory function. If persistent sleep disturbances occur, it is prudent to seek medical guidance for therapeutic interventions such as diazepam and Zopiclone. In daily life, you should pay attention to your diet, avoid spicy foods, including scallions, ginger, etc., and eat more nutritious foods such as milk and eggs. In addition, proper aerobic exercise before bed, such as yoga or jogging, can be used as an effective way to improve sleep disorders and improve overall sleep quality.

3. Microscopic studies on neuroplasticity and sleep

In previous studies, neuroplasticity can be explained and acted on through these aspects. First, the chronology of the neurophysiological mechanisms implementing neuroplasticity ranges from short-term, transient synaptic strength amendments to longer-term, structural alterations.[3] Second, these components make up the molecular architecture and process of neuroplasticity. Neuroplasticity is facilitated by molecular processes such as synaptogenesis (the establishment of freshly developed synaptic connections), neurogenesis (the proliferation of new neurons), angiogenesis (the formation of new blood vessels), and gliogenesis (the production of non-neuronal glial cells from within the brain).[4] Additionally, the following is a theoretical description of neuroplasticity's biological structure: Presynaptic changes such as increased neurotransmitter and neurotrophin discharge from the presynaptic neuron, went down neurotransmitter reintroduction and breakdown in the cleft between synaptic neurons, and postsynaptic adaptations that bring about the deposition of extra neurotransmitter receptors on the membrane of the cell are examples of cellular adaptations in response to sensory experience[5].

Table 1. Neurophysiological and molecular measures of neuroplasticity.

Measure	Method	Protocol	Interpretation
MEP	TMS	Single-pulse TMS over M1	Corticospinal excitability
RMT	TMS	Single-pulse TMS over M1	Minimum stimulation intensity required to evoke an MEP
CSP	TMS	Single-pulse TMS over M1 during muscle activity	Intracortical and spinal excitability Suppression of the contralateral target muscle
SICI	TMS	Paired-pulse TMS over M1: a subthreshold stimulus in advance (1-6ms) of a suprathreshold stimulus	Cortical suppression of MEP amplitude
LICI	TMS	Paired-pulse TMS over M1: a suprathreshold stimulus followed (50-200ms) by a suprathreshold stimulus	Cortical suppression of MEP amplitude
ICF	TMS	Paired-pulse TMS over M1: a subthreshold stimulus in advance of a subthreshold stimulus (8-30ms)	Cortical suppression of MEP amplitude
SAI	TMS and PNS	single-pulse TMS over M1 preceded by PNS stimulation (20-25ms)	Cortical suppression of MEP amplitude reflecting sensorimotor integration Plasticity-including TMS paradigm leading to lasting increases in corticospinal excitability
rTMS	TMS	Rapid(5-10Hz) succession of pulses	Plasticity-including TMS paradigm leading to lasting increases in corticospinal excitability
cTBS	TMS	Clusters of three TMS pulses delivered in rapid succession	Plasticity-inducing rTMS paradigm resulting in LTD and cortical excitability suppression
PAS	TMS and PNS	Repeated pairings of TMS and PNS pulses delivered in rapid succession (50-150 pairings)	Plasticity-inducing paradigm to strengthen corticospinal excitability
ERP	EEG	Latency and amplitude of cortical activity evoked by exposure to repeated stimuli such as lights or audible tones	Sensory processing and cognitive functioning
Cerebral blood flow	TCD	Recorded from cerebral arteries to measure rate and velocity of blood flow in the brain	Hemodynamic activity which facilitates neurovascular coupling and brain function
BDNF	Blood draw	Blood serum or plasma levels measured using ELISA	Concentration of circulating BDNF which promotes neuroplasticity and memory
IGF-1	Blood draw	Blood serum or plasma levels measured using ELISA	Concentration of circulating IGF-1 which promotes brain development and maintenance
VEGF	Blood draw	Blood serum or plasma levels measured using ELISA	Concentration of circulating VEGF which promotes neural growth and proliferation

BDNF, brain-derived neurotrophic factor; CSP, contralateral silent period; cTBS, continuous theta-burst stimulation; EEG, electroencephalography; ELISA, enzyme-linked immunosorbent assay; ERP, event-related potential; ICF intracortical facilitation; IGF-1, insulin-like growth factor 1; LICI: long-interval intracortical inhibition; M1, primary motor cortex; MEP, motor-evoked potential; PAS, paired-associative stimulation; PNS, peripheral electrical nerve stimulation; RMT, resting motor threshold; rTMS, repetitive TMS; SAI, short-latency afferent inhibition; SICI, short-interval intracortical inhibition; TCD, transcranial doppler, VEGF, vascular endothelial growth factor[6].

Sleep deprivation affects the ability to regulate emotions and causes cognitive decline by reducing the synaptic plasticity of the prefrontal limbic system. Moreover, the loss of synaptic plasticity is linked to three separate types of structural and molecular dysfunction, including reversible blood-brain barrier malfunction, a functional breakdown of neurotransmitter systems, and uneven electrolyte distribution.[7]

4. Research on other influencing factors

4.1. Exercise

Exercise promotes increased neuronal activity throughout the prefrontal limbic system, especially when undertaken at moderate intensity levels. This increase in brain activity supports improved emotional control as well as cognitive function [7]. In order to deal with sleep loss, aerobic exercise is believed to have neuroprotective impact on the microglia-mediated pruning of synaptic connections that takes place in the sleep-deprived brain [8]. Therefore, incorporating aerobic exercise into your daily routine can serve as a valuable strategy for mitigating the adverse effects of sleep deprivation. The cumulative findings and conclusions of these studies highlight the positive effects of sustained daily physical activity on enhancing cortical neuroplasticity. This means that intelligently engaging in physical activity can serve as a means of enhancing neurogenesis and facilitating the construction and repair of synaptic connections, while keeping sleep quality unaffected.

4.2. Diet

Diet is a lifestyle decision that has been demonstrated that it has an impact on brain chemistry and function, which has the potential to alter how neuroplastic the brain is [9,10]. Nutritional therapies that have an impact on cognition and mood consist of calorie restriction, intermittent fasting, as well food supplementation [10].

This intricate web of dietary-sleep interrelationships is exemplified by a comprehensive graduate study that delved into the ramifications of the ketogenic diet on morbidly obese adolescents. The research within demonstrates an intriguing connection between eating habits and the world of sleep. Unexpectedly, the dietary change that caused significant weight loss also resulted in a notable increase in the duration of rapid eye movement (REM) sleep and a discernible decrease in non-rapid eye movement (non-REM) sleep, thereby successfully reestablishing normal sleep patterns. This remarkable change is linked by scientists to the reduction of airway constriction within the sleep cycle, highlighting the complex interaction between eating habits and sleep dynamics [11].

In summation, it is incontrovertibly apparent that the meticulous curation of a health-conscious dietary regimen can wield a salubrious influence on the qualitative attributes of our sleep, thereby potentiating the brain's intrinsic capacity for neuroplasticity. Thus, the synergistic confluence of diet and sleep emerges as a profound axis through which we may harness and augment the brain's innate adaptive prowess.

5. Discussion

Based on previous experimental studies and related literature, this paper summarizes the macro and micro understanding of the impact of sleep on neuroplasticity, 1 combining the single factor of sleep with the influence of multiple factors such as environment and diet on neuroplasticity. This paper summarizes and reflects on some relevant theoretical basis: under macro conditions, especially in the state of sleep deprivation, people's cerebral blood oxygen is reduced, and neurogenesis and synaptic

formation are reduced in the state of depression, which shorten the attention span and reaction speed, and even lead to the occurrence of wrong reactions. At the same time, less sleep was frequently connected with improved memory. The findings of linked studies and tests revealed diminished synaptic plasticity in the prefrontal limbic system, impoverished neurotransmitter system function, unequal electrolyte distribution, and reversible blood-brain barrier malfunction. At the same time, sleep is also related to environmental factors such as diet and exercise. Combined with the three, reasonable and effective physical exercise and healthy and appropriate eating habits can help establish high-quality sleep quality, provide protection for us to form healthy and efficient sleep, and then help promote the occurrence of neuroplasticity.

Under the social reality of poor sleep quality and the general reduction of sleep market, the health problems related to sleep have gradually received everyone's attention, and caused related social hot issues. Under such a background, this review study has enlightenment and practical value from multiple perspectives.

One is multi-factor and realistic treatment intervention. Studies of influencing factors such as exercise and diet highlight the importance of taking a holistic approach to health and the complexity of multiple environmental factors in reality. It recognizes that sleep is not isolated from other aspects of health, but rather interlinked with lifestyle choices. This holistic viewpoint is in line with contemporary medical practice, which seeks to deal with the underlying causes of health issues while also acknowledging that people differ in their sleep needs as well as responses to lifestyle factors. This perspective also allows for a more thorough comprehension of the mechanisms by which exercise and diet affect sleep, as well as a recognition that neuroplasticity may have potential applications in enhancing general health. For example, individuals, healthcare professionals, and public health policymakers can use this knowledge to develop interventions and recommendations tailored to the individual level. Healthcare providers can work with patients to create customized plans to meet their unique sleep, exercise, and dietary requirements, promoting better sleep and cognitive function and optimizing their cognitive and health levels.

The second is prevention and recovery of health. The findings provide insights into preventative measures for cognitive decline and neurodegenerative diseases. Individuals may decrease their chance of contracting illnesses like Alzheimer's by encouraging regular physical activity and a balanced diet. This proactive strategy may result in more affordable long-term healthcare expenses and enhanced the long-term health outcomes. At the same time, in addition to preventing cognitive decline, this study suggests that lifestyle factors can positively enhance cognitive function. Individuals seeking to improve attention, memory, and overall cognitive performance can incorporate evidence-based practices into their daily lives, potentially improving productivity and quality of life.

Third, promote disciplinary cooperation and public health policies. Research into the interactions between sleep, exercise, diet, and neuroplasticity encourages interdisciplinary collaboration between fields such as neuroscience, nutrition, psychology, and public health. This collaboration could lead to a more complete understanding of the complex relationship between lifestyle choices and brain health. Governments and healthcare organizations can use this knowledge to develop policies that promote healthier living environments, which could include increasing access to exercise facilities, promoting healthier dietary choices, and raising awareness about the importance of sleep.

In conclusion, the study of influencing factors such as exercise and diet provide valuable insights into how individuals can optimize their cognitive health through lifestyle choices. It provides practical strategies for preventing cognitive decline and enhancing cognitive function, which can benefit individuals, healthcare systems, and society as a whole. By recognizing the interconnections between sleep and other aspects of health, this research paves the way for a more comprehensive and proactive approach to health promotion and disease prevention.

6. Conclusion

This paper summarizes the macro and micro understanding of the influence of sleep on neuroplasticity, and also combines the single factor of sleep with the influence of multiple factors such as exercise and

diet. There are three conclusions: First, sleep loss will cause a decline in attention and memory, and even lead to the occurrence of wrong reactions. The second is that sleep deprivation impairs neurotransmitter system performance, causes uneven electrolyte distribution, and causes reversible blood-brain barrier malfunction, all of which diminish the prefrontal limbic system's synaptic plasticity. Third, sleep is closely related to environmental factors such as diet and exercise. Reasonable physical exercise and healthy eating habits can help establish high-quality sleep quality and help promote the occurrence of neuroplasticity.

However, it is worth noting that this paper still has some limitations. First, the article does not describe each mechanism in detail, but ties each study together to summarize cognition from a larger perspective. This form lacks detailed scrutiny and can be a bit jumpy and difficult to understand. Secondly, this paper lacks its own experimental data and relevant clinical data, and does not provide detailed examples and treatment perspectives to verify whether some conclusions can be completely correlated, which may lead to certain errors or one-sidedness of the conclusions. Third, this article only deals with exercise and eating habits, which is incomplete, and looking at more environmental factors may provide more novel perspectives on the relationship between neuroplasticity and sleep.

In future research, there are two possible expectations: one is to add more clinical data or experimental tests on primates to explore more microscopic brain structures, which can deepen the understanding of the correlation between sleep and neuroplasticity, and provide more molecular biological mechanisms and theories. The second is to find more research angles, not only in terms of exercise and diet, but also in terms of psychological states or environmental atmosphere, such as sleep music, white noise and so on.

In summary, this paper summarizes the mechanism of sleep on neuroplasticity and the correlation between the two from macro and micro perspectives, combining the effects of exercise and diet on sleep and neuroplasticity. The interactions between the organism and its environment have gained greater recognition thanks to these summaries. This can provide a neurobiological basis for the study of brain physiological structure and sleep research, and even provide new therapeutic ideas for the clinical treatment of some neurological diseases, and influence the government or relevant institutions to formulate sleep guidance policies to improve the quality of life and health of contemporary people.

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The application of gene editing in treating cancer

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Abstract. Current gene editing technology mainly involves inserting, replacing or deleting DNA sequences to change the genetic material of an organism, and its most common aspects are using this technology to mass-produce some drugs, such as insulin, or using gene editing to change some nutrients in plants to help others. This article use a literature review and, expects to give an extensive outline of the utilizations of quality altering in medication, with a specific spotlight on its utilization in cancer disease treatment. The paper finds that gene editing technology has emerged as a powerful tool in the field of medicine, revolutionizing the treatment of diseases, including cancer and some systemic diseases. There are many different diseases in the world that currently have no cure, because medicine needs to go through many stages before it can be brought to market and given to people, but through gene editing technology can greatly help alleviate or even reverse these diseases. Other than that, this research deepens our understanding of biology, thereby contributing to the future connection between biotechnology development and medicine, and helping more patients with difficult and complex diseases.

Keywords: Ene editing, medicine, cancer, DNA sequence, CRISPR.

1. Introduction

The technology of gene editing is not as common in today's society as ordinary drugs, but if the technology grows and develops to a certain stage, it may lead humanity to a new stage. In today's society, many drugs take a decade or more to research, and need to go through layers of testing before they are finally used to treat major diseases. But now there are many difficult and complex diseases in society that cannot be cured by drugs, such as cancer or systemic diseases or a series of serious diseases such as leukemia. Cancer is caused by changes in the genome of tumor cells. So in gene editing technology there is a technology called CRISPR/Cas9 gene editing technology, which can achieve precision gene editing, and then edit the genome to explore the mechanism of tumor development and development. This procedure involves just complex nuclease proteins and short RNA as site-explicit endonuclease catalysts and can target practically any genomic site. Lately, this framework has been progressively applied to disease exploration and treatment and has accomplished astounding outcomes.

Using the research method of Literature review, this paper mainly studies how gene editing technology is developed and applied in cancer research and treatment, and further studies what convenience CRISPR/Cas9 technology provides for cancer research and treatment and some of its effects. Whether the study of this tool can effectively prevent or treat cancer in the near future.

The main significance of this article is to do some deeper research on gene editing and CRISPR/Cas9 technology. It also provides more ideas and some recommendations for the treatment of cancer, in

addition to allowing more people to understand and know the application of gene editing technology in medicine and the effective treatment of diseases.

2. Introduction to gene editing therapy

As a new science and technology, gene editing technology involves a wide range, and has become a more popular new technology in the field of biology [1]. Gene therapy mostly involves replacing defective genes adding new genes or modifying gene sequences in an endeavor to fix sicknesses or improving the body's ability to fight disease, or using artificial nuclease technology to add and splice biological genes. This technology is the innovative frontier of contemporary life science technology. With the continuous growth of gene editing technology systems, human beings can have more high-end technology to fix sicknesses and facilitate all aspects of the world. Quality treatment holds guarantee for a great many infections, including malignant growth, coronary illness, diabetes, hemophilia and AIDS. CRISPR-Cas9 innovation is a novel and profoundly exact quality altering instrument that is changing disease examination and treatment. Scientists are utilizing this innovation to concentrate on how disease develops and track down new possible restorative systems, and this treatment is likewise going through clinical preliminaries in malignant growth patients. Conventional quality altering innovation depends on the standard of DNA homologous recombination, utilizing outer benefactor quality pieces to take out or add target quality parts. The main principle of genome editing technology refers to the addition, deletion, decoration or substitution of specific gene fragments in living organisms, so the predetermined DNA succession is changed into the normal genome arrangement, lastly the quality sections are altered or even make hereditary impacts [1]. At the same time, gene editing has many ethical problems. For example, the most well-known gene-edited baby event in 2018 mainly involved an associate professor at the Southern University of Science and Technology who genetically edited a pair of newborn babies to make them naturally resistant to HIV after birth. This incident has aroused a wide range of academic and public discussions around the world. In recent years, gene editing technology has played an advantage in the fields of human disease treatment, health care, the food industry, and the crop industry. However, while bringing welfare to human beings, it also inevitably has huge ethical risks. The future promotion and development of this technology will be closely related to everyone. By and large, quality altering advances principally incorporate zinc finger nuclease, record activator-like impact factor nuclease and grouped consistently blended short palindromic rehash succession (CRISPR) innovation. In addition to gene editing for cancer, insulin was first discovered in the pancreas of cows and pigs, but it takes a lot of pancreas to make a few milligrams of insulin. However, through gene editing technology, insulin can be produced in large quantities to meet the needs of patients. All you need to do is to cut the gene fragment that normally produces insulin in the human body into the bacteria so that the rapid reproduction of the bacteria can quickly obtain a large amount of insulin, which is very efficient and convenient. In terms of human somatic cells, gene editing technology can deepen people's understanding of the molecular process that controls the occurrence and progression of diseases. Further understanding of gene editing technology carried out in laboratories on human cells, tissues, embryos and gametes provides an important way to further understand human gene function, DNA repair mechanism, and the link between genes and diseases. Cancer spread and the relationship between genetic diseases and genes. Basic research on gene editing technology could also improve human understanding of mammalian reproductive and developmental processes. Studying human germ cells can also provide a detailed understanding of human development and fertility issues, thereby bringing fertility possibilities to people who are not easily pregnant [1]. Although gene editing has made breakthrough progress in the treatment of hereditary sicknesses, cancer, infectious diseases, etc., the editing of germ cells and embryonic cells has always been controversial, and the ethical issues of gene editing are also one of the factors limiting its application [2]. The emergence of gene editing greatly facilitates medical research and treatment, and the cost is also greatly reduced compared to the development of medicine [3].

3. The Application of Gene Editing in Cancer

Cancer is an unmanageable sickness with a high death rate, which has drawn in worldwide consideration. Dangerous growths are answerable for one out of six passings around the world, undermining large number of lives. Notwithstanding many energizing accomplishments in the field of disease therapy, including a medical procedure, radiation treatment, chemotherapy, designated biotherapy, and new blend treatments, high postoperative repeat rates, radiation/chemotherapy obstruction, and hurtful poisonous secondary effects remain hindrances to endurance and survival [4]. This prompts the turn of events and examination of CRISPR/Cas9 innovation. Utilizing CRISPR/Cas9 to take apart compound hereditary connections uncovers how disease responds to pharmacological treatment, which is a significant utilization of the technology [5]. CRISPR-Cas9 framework is a versatile invulnerable system that utilizes bacterial archaea to guard against unfamiliar nucleic acids [1]. The CRISPR genome altering framework perceives a particular DNA grouping by the objective succession carried on the sgRNA, which makes the CRISPR framework exceptionally unambiguous. The utilization of this innovation can be utilized to fix the quality change locales of sicknesses or to accomplish the capability loss of significant qualities. As of now, this innovation has been effectively applied to the treatment of an assortment of hereditary diseases [6]. To shield themselves from trespassers, for example, infections, these microorganisms catch sections of the intruders' DNA and store them as parts called CRISPR, or short palindromic rehashes that consistently group together. On the off chance that similar microorganisms attempts to go after once more, these bits of DNA (which become short bits of RNA) help a chemical called Cas find and cut the trespasser's DNA. In the lab, CRISPR apparatuses comprise of two fundamental jobs: guide RNA and DNA-cutting catalysts, most regularly Cas9. The researchers planned the aide RNA to mirror the DNA of the quality to be altered, called the objective quality. The aide RNA helps out the Cas and guides the Cas to the objective. At the point when the aide RNA matches the DNA of the objective quality, the Cas cuts the DNA [3].

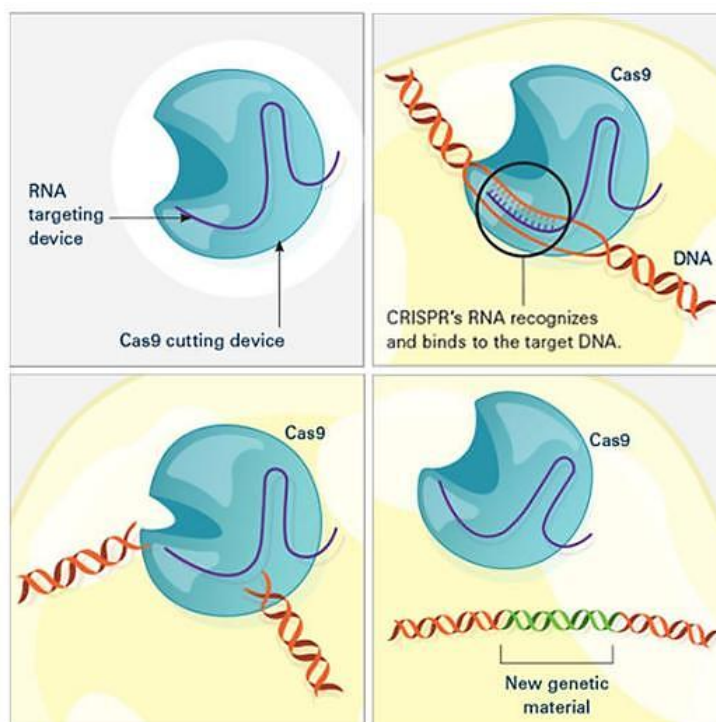


Figure 1. CRISPR consists of a guide RNA and the Cas enzyme [3].

Through CRISPR-based vast screening, a quality need dataset for 14 human AML cell lines was produced and an overall methodology for characterizing mammalian quality organizations and

engineered deadly connections was proposed [2]. Cas9-intervened genome altering has three necessities: (1) Single guide RNA (sgRNA) grouping, the CRISPR/Cas9 framework is the main CRISPR/Cas framework for genome altering, to some extent since it contains a related, effectively programmable sgRNA. It's only 20 nucleotides long. sgRNA consists of CRISPR RNA (crRNA) that has a mutually complementary sequence with a target site, and trans-activating crRNA (trans-activating crRNA, tracrRNA) is grouped into [1,7]. (2) There is a Cas9 protein with a nuclear localization signal. In order to perform genome editing function, the CRISPR/Cas9 system also needs a keying enzyme closely related to the two RNA groups, Cas9 ribonuclease. These RNA are expected to direct the Cas9 protein to the objective site and initiate the Cas9 nuclease. (3) Protospacer contiguous theme (PAM), sgRNA and Cas9 opal intersection to frame complex, sgRNA and Cas9 opal intersection to shape complex, and rigorously distinguish the objective reciprocal DN-A succession situated close to the 3' terminal horizontal wing of the PIN, which is generally made out of NGG or Bother (N can be A, T, G or C). Then, at that point, DNA twofold strand breaks (DSBs) are triggered [7]. Tumorigenesis is a perplexing interaction including multi-quality and multi-stage changes, including initiation of proto-oncogenes, inactivation of cancer silencer qualities, microdeletions, micro repeats, rearrangements and structural abnormalities of chromosomes. Immunotherapy is one of the main forms of cancer treatment, which uses monoclonal antibodies or donor T cells with anti-tumor function to target the autoimmune system [2]. The most recent progressions have assisted with tending to one of the unmistakable worries about this technique which is the off-target mixes saw with dsDNA and have brought about additional examinations being completed for possibly more secure and more designated quality treatment, to make it accessible for the clinical preliminaries to successfully treat cancer [6].

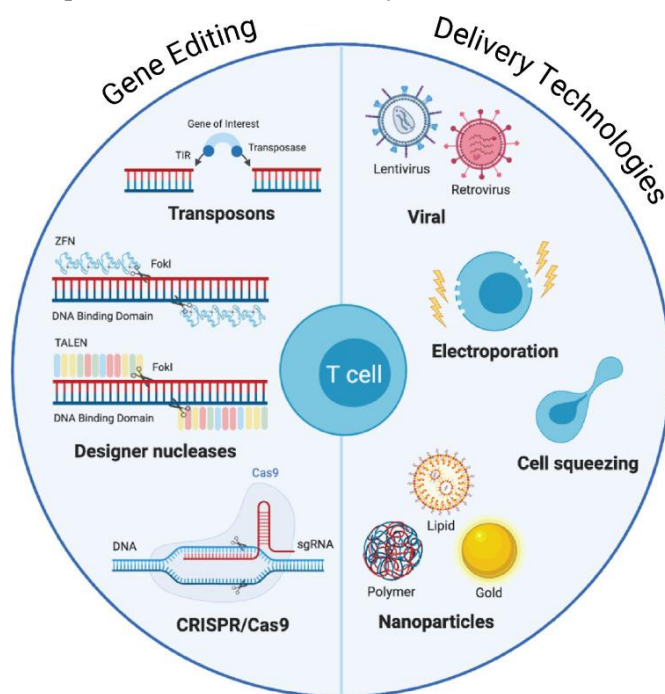


Figure 2. Delivery technologies for gene editing of T cells. Figure was created by the authors with BioRender.com [8].

Quality altering systems that have been investigated in White blood cells for applications in disease immunotherapy incorporate transposons, planner nucleases like zinc finger nucleases (ZFN) record activator-like effector nucleases (TALEN), and grouped routinely interspaced short palindromic rehashes (CRISPR)/Cas9. Notwithstanding popular transduction, novel conveyance frameworks, for example, electroporation, cell crushing, and nanoparticles — have been used in new immunotherapy methodologies to additional improve restorative efficacy [9].

4. Conclusion

The research conclusion of this article is that the edited technology has made great achievements in the treatment of cancer, but it is in progress, and it has not been fully implemented into the real treatment stage, and more is in the experiment. CRISPR-Cas9 technology, for example, has made it easier to study cancer and its treatments and has made great academic breakthroughs, but it also faces huge ethical problems [10]. At the same time, it also provides new ideas for solving some diseases that cannot be solved by traditional therapies. In the future, many emerging technologies will be combined in clinical medicine practice, and it is believed that in the near future, CRISPR-Cas9 technology can be applied to real treatments, so as to help more patients avoid some problems similar to cancer or systemic diseases. There are also many shortcomings in this study. For example, the study in this paper may not be thorough enough in some aspects to give a very clear explanation. In the future, further studies will be conducted in this aspect to integrate various emerging technologies more closely with clinical medicine. All the ethical and moral issues of this technology are not elaborated in detail in this article, and more research and investigation will be done to improve this research.

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Investigation of the causes of adolescent lumbar and shoulder-neck diseases and related mitigation approaches

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Abstract. With the rapid development of global economy and information technology, people's lifestyle has undergone great changes, and the prevalence of neck and shoulder pain and low back pain has also increased, and the age of onset is trending younger, which seriously affect their study and life, resulting in greater family burden. The survey shows that in recent years, the incidence of pain has increased year by year due to the high learning pressure of teenagers, playing mobile phones and computers for a long time. Teenagers' long-term incorrect sitting position, sleeping position, improper pen use and other bad living habits, mental and psychological factors may eventually induce the pain through affecting biomechanical imbalance. The author has discussed the etiology, intervention and also the prevention of general neck and shoulder pain and low back pain in order to provide more theoretical basis for the management of this pain in the future and better guide the clinic.

Keywords: Musculoskeletal pain, neck and shoulder pain, low back pain.

1. Introduction

Musculoskeletal pain (MSP) is a public health problem for both men and women at all ages, especially adolescents. Studies have shown that the prevalence of neck and shoulder pain (NSP) and low back pain (LBP) in adolescents is on the rise and worsens with age. And is closely related to poor mental state, vulnerable to psychological factors, living habits and other factors, may also be an important factor leading to adult cervical and lumbar diseases, continue to the work stage, so it is urgent to understand its potential risk factors and implement effective prevention and intervention measures. This study explored the influence and intervention strategies of adolescent patients with neck, shoulder and back pain [1].

2. Risk factors of the NSP

The generalized neck and shoulder pain which is the common and shared symptom of many diseases include all of the discomfort in the neck and shoulder. The development of neck and shoulder pain is due to the combined effect of multiple factors of which demographic factors, disease factors individual living habits are more common while job-related factors and psychosocial factors in the current etiology study has become a hot spot. However the etiology study should be expanded widely and deeply for

example the joint the effect of related genes the synergistic action of the factors and the multi a rate analysis in the etiology study, all of which should be paid more attention.

2.1. Demographic factors

Numerous studies have shown that women are more prone to neck and shoulder pain. The research of Fredriksson has also confirmed that women are more prone to neck and shoulder pain than men by case control study [2]. Sui'an Liang and others reported that the incidence rate of periarthritis of shoulder in women was significantly higher than men [3]. It have shown that women are a risk factor for neck and shoulder pain from both cross-sectional and longitudinal cohort studies

2.2. Disease factors

Domestic scholars generally believe that neck and shoulder pain is a type of clinical manifestation , especially cervical spondylosis, shoulder periarthritis, and tissue inflammation around the neck and shoulders. Yongxi liu and others have also reported heart disease characterized by neck and shoulder pain is similar to Vogt 's [4]. According to the survey, it shows that which is also a risk factor of a history of rheumatism, heart disease, angina.

2.3. Individual living habits

Personal lifestyle has a significant impact for diseases. It is the most basic requirement for preventing diseases by advocating for a healthy lifestyle. Bad Lifestyles such as smoking, excessive drinking, frequent staying up late, and lack of exercise are common risk factors for multiple diseases.

2.4. Job-related factors

The main focus of research on the occurrence of neck and shoulder pain and work-related factors in which included terms of job type, work style, work related physiological factors, etc. Háles believe that specific physiological factors include frequent, repeated or sustained exertion, carrying a heavy load , prolonged maintenance of a fixed position, insufficient rest time, vibration, and low-temperature environment [5].

2.5. Psychosocial factors

Social psychological factors are ubiquitous and can guide in the social environment The various social factors that cause psychological stress and thus affect health The study of the impact on health began around the 1920s with the "Heart Physical medicine "mainly studies the effects of psychological and social factors on health and illness.

The role of diseases and their interrelationships, including personality psychology characteristics, emotions, stress, and life events

Sklgate believe that life events and significant changes in life are important risk factors for neck and shoulder pain [6].

Neck and shoulder pain is both a clinical syndrome, according to the definition of psychosomatic diseases in Occupational Health and Occupational Medicine should belong to the category of psychosomatic diseases. The causes of neck and shoulder pain are diverse, and it is a combination of personal, professional, socio psychological, and other factors. The result of cooperation. Therefore, in the future, the prevention of neck and shoulder pain should be "tailored to the disease", and comprehensive and targeted preventive interventions should be carried out to achieve ideal results.

3. Intervention of the NSP

The purpose of the neck health care is mainly to exercise the head self-weight load and neck muscle strength, so as to improve the stability of the cervical spine. During exercise, stretching the muscles may also enhance the contraction of the neck muscles. Neck exercise will not be restricted by the region, and cervical spine function can be adjusted in this way at work, study or at home. A randomized controlled study included 50 adolescent patients with shoulder and neck pain, randomly divided into two groups,

25 cases each. The study group used neck health exercises combined with exercise intervention, and the control group used neck health exercises to intervene. VAS pain score, cervical dysfunction index, MF slope, MPF slope, MVC improvement of right upper trapezius muscle and other functional recovery were compared between two groups after intervention. The results showed that the VAS pain score was significantly decreased in both groups, with the average score of 2.80 in the study group and 3.56 in the control group ($P<0.05$). The average score of cervical spine dysfunction index was 23.05 in the study group and 31.06 in the control group ($P<0.05$). The MF slope and MPF slope of the study group were -0.06 and -0.31, respectively, which were better than those of the control group ($P<0.05$). The improvement of MVC of right superior trapezius muscle in study group was better than that in control group, and the difference was significant ($P<0.05$) [7].

4. Risk factors of the LBP

4.1. Poor posture.

One significant pathogenic factor of adolescent lower back and shoulder-neck diseases is poor posture. Many adolescents adopt improper sitting or standing positions for prolonged periods, which places strain on the muscles, tendons, and ligaments in the lower back and shoulder area. Slouching or hunching forward while using electronic devices or engaging in study activities can lead to abnormal stress on the spine and its surrounding structures. This constant strain on the musculoskeletal system can contribute to the development of pain and discomfort in these regions.

4.2. Sedentary lifestyle

Another contributing factor to adolescent lower back and shoulder-neck diseases is a sedentary lifestyle. Many adolescents nowadays lead increasingly sedentary lives, lacking sufficient physical activity and spending prolonged periods sitting. This sedentary behavior results in muscle imbalances and weakens the muscles that support the lower back and shoulder-neck areas. Insufficient movement leads to decreased blood circulation, oxygen supply, and nutrition in these regions, further exacerbating the risk of developing pain and discomfort in the shoulders, neck, and lower back.

4.3. Physical load

Physical load is a risk factor for low back pain, which can cause serious consequences in adolescents. Neck pain is more influenced by psychosomatic factors, while shoulder and back pain are closely related to physical factors. However, some studies have pointed out that backpack weight is not related to the occurrence of LBP. Single-factor studies have shown that appropriate weight bearing can reduce the incidence of NSP/LBP. After multivariate analysis, the correlation between overloading and NSP disappeared, but the correlation with LBP remained. Because the travel time is often longer, the positive effects of loading may be underestimated, and excessive load and fatigue caused by too long show a significant correlation [8].

4.4. Mental factors

Mental factors can cause different degree of sensitivity to pain, and good mental state can reduce the incidence of LBP. Mental factors are more likely to cause LBP than physical factors. The onset of NSP is associated with depressive states, especially in girls. Teenagers are susceptible to more negative factors in life, which mainly come from teachers, parents, classmates and themselves. Adolescents bear a great deal of mental stress, which plays a small role in the occurrence of neck, shoulder and low back pain and this also explains why the incidence of adolescents who have confidence in themselves is reduced [9].

4.5. Others

Additionally, the excessive use of digital devices has emerged as a notable pathogenic factor in the development of lower back and shoulder-neck diseases among adolescents. Extended periods of looking

down at screens create repetitive strain on the neck muscles and contribute to poor posture. This recurring stress on the cervical spine increases the risk of developing shoulder-neck diseases, such as cervical spondylosis. The widespread use of electronic devices among adolescents further compounds the impact of this pathogenic factor.

5. Intervention the LBP

5.1. Medication

The first point is drug treatment. Taking anti-inflammatory drugs, taking injections, and applying ointments are all considered drug treatments. Drug treatment mainly aims to alleviate and target mild symptoms. After reading about the treatment of lumbar disc herniation symptoms with traditional Chinese and Western medicine, the article divided the patients into three groups: the treatment with *Codonopsis pilosula* and the treatment with Wushao snake centipede [10]. The patient was 60 years old and had long-term lumbar disease. He had received Western medicine treatment but did not improve. In this experiment, *Eucommia ulmoides*, centipede, cassia twig, and scorpion were added. After taking it continuously for 2 weeks, the pain decreased. After persisting in taking the medication for more than a month, the pain disappeared and the waist moved freely. So it can be concluded that Western medicine cannot provide a good treatment effect or solution for long-term chronic injuries. In addition, the comprehensive treatment of lumbar disc herniation with traditional Chinese medicine has many advantages: good treatment effect, simpler and more feasible than surgical treatment, less pain, low risk, and low cost. Treatment and care based on syndrome differentiation and treatment are easy for patients to accept, and their concerns are significantly reduced. Conclusion: The combination of traditional Chinese and Western medicine in the treatment of lumbar disc herniation has its own characteristics, and it should be combined with traditional Chinese and Western medicine to achieve good therapeutic effects. In the non-acute phase, it is recommended to strengthen lumbar functional exercise and build more lumbar muscle.

5.2. Physical therapy

On the other hands, physical therapy is good choice, physical therapy mainly uses acupuncture and moxibustion, massage, and electrotherapy to dredge blood and remove blood stasis. In physical therapy, Chinese medicine mainly advocates acupuncture and moxibustion therapy, which can also combine acupuncture and moxibustion and massage to achieve a better effect. In acupuncture and moxibustion therapy, Chinese medicine has divided the low back pain into very detailed reasons, including not only rheumatic low back pain, cold and wet low back pain, but also damp heat low back pain, congestive low back pain, kidney deficiency low back pain, etc. The actual group suffering from low back pain is mostly characterized by deficiency and excess, especially in cases of cold dampness and congestive low back pain. Currently, there are many methods for treating low back pain, such as medication and physical therapy. The patients have different causes and treatment plans, but the effective way of speaking and acting is still acupuncture and moxibustion therapy. Different acupoints in the treatment method can have different therapeutic effects. We should choose Shenshu, Yaoyangguan, Guanyuanshu, Weizhong and Dachangshu for treatment, of which acupuncture and moxibustion at Shenshu has the effect of removing cold and dampness; Yaoyangguan is a local acupoint selection mainly used to clear local meridians and achieve pain relief; Guanyuan Shu and Dajie Shu have the effects of dispelling wind, dispersing cold, clearing collaterals, and relieving pain; The main purpose of selecting acupoints along the Wei Zhong[8] meridian is to dredge the bladder meridian qi. And in acupuncture and moxibustion, adding massage manipulation can effectively shorten the course of treatment and increase the therapeutic effect. Traditional Chinese medicine believes that there are close connections in the tissues of the human body, and diseases also have a state of mutual influence. In addition, stimulation of local inflammation can eliminate and alleviate the development of inflammation. Physical therapy still belongs to conservative treatment with minimal harm and side effects on patients, and can be very effective in

treating mild lumbar diseases and lumbar soft tissue contusions and chronic diseases caused by different factors [11].

5.3. *Surgical therapy*

Surgical therapy, as a treatment method that can achieve the maximum benefits in the shortest possible time, is usually targeted at patients with moderate to severe symptoms. Surgical treatment often has personalized adjustments and adopts different surgical plans according to the patient's different symptoms to achieve the best therapeutic effect. Currently, effective surgical treatment plans include but are not limited to traditional lumbar discectomy surgery methods, including posterior approach, intervertebral fenestration surgery Hemilaminectomy, total laminectomy, nucleus pulposus removal, anterior spinal approach. Which method to choose, such as nucleus pulposus removal or nucleus pulposus removal combined with intervertebral fusion surgery. This mainly depends on the condition of the lesion and the experience of the surgeon. It is currently recognized that the indications for surgery are when symptoms are severe and when 242100conservative treatment is ineffective for six to eight weeks, and there is a widespread feeling of muscle paralysis, requiring surgical treatment. Although surgical treatment can minimize and quickly alleviate symptoms, it is still necessary to pay attention to many concurrent symptoms and follow-up treatment, such as preoperative mispositioning and unfamiliar anatomical structures. After surgery, there were nerve damage, dural tear, vascular and visceral injuries [12].

6. The prevention of the NSP and LBP

Regular physical exercise and physical therapy are important approaches to address sedentary-related issues. Engaging in exercises that target the muscles of the shoulders, neck, and lower back can help strengthen them and improve flexibility. Physical therapy modalities like heat or cold therapy, ultrasound, and electrical stimulation can further reduce inflammation, promote healing, and provide pain relief.

Ergonomic adjustments also play a crucial role in preventing and managing these issues. Maintaining correct posture, using ergonomic chairs and desks, and positioning screens at eye level can reduce strain on these areas. Adolescents should be encouraged to adopt proper ergonomics to prevent any long-term negative consequences of sedentary behavior.

Regular breaks and movement are essential in alleviating discomfort caused by prolonged sitting. Short breaks that involve stretching exercises or walking can relieve muscle tension, improve blood flow, and reduce the risk of developing musculoskeletal disorders. Incorporating furniture and equipment that promote dynamic sitting or active movement can also be beneficial.

7. Conclusion

NSP and LBP of adolescents is a very common life problem, especially more and more children use electronic products and sit for a long time to study, we must actively face these pain, early intervention, early prevention, do not let the existence of pain seriously affect their normal life, otherwise it will lead to serious complications. At the same time, it is necessary to strengthen the prevention of risk factors and reduce the occurrence of pain. Clinically, it is necessary to further increase evidence-based research, so that more adolescents are aware of this serious problem and pay more attention to it psychologically. At the same time, parents should also pay more attention to their children and find problems in time. So that teenagers may grow up more healthily.

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MRI and CT for scanning human bodies

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Abstract. Prostate-Specific antigen (PSA) is one of the most popular biomarkers for prostate carcinoma (PCA) at present. PSA indicates initial cancer and recurrence following therapy, but does not distinguish clearly from local or remote lesions. Molecular and functional scans, which can give a thorough and complete picture of PCR amplification, are a more robust instrument for detecting and assessing the progression of cancer at the stage as well as at subsequent stages. In this paper, PET or CT, MRI are useful for diagnosing, staging and regrading of PCA, as well as their application to prognostic, therapeutic and therapeutic evaluation. Novel imaging techniques, including novel radiotracers and PET/MRI, are also presented in this paper. Looking forward, the evolution of imaging techniques holds promise for improved PCA management. Continued innovation in radiotracers and hybrid imaging technologies such as PET/MRI could revolutionize early detection, accurate staging, and effective treatment, paving the way for precision oncology in prostate cancer.

Keywords: CT, MRI, PSA, PCA

1. Introduction

In recent decades, the landscape of medical imaging has witnessed a profound transformation thanks to pioneering technologies such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). These remarkable modalities, introduced in the late 1970s and 80s, have redefined the practice of medicine by bestowing healthcare professionals with powerful tools for diagnosis, treatment, and improved patient outcomes. Within prostate cancer (PCa) management, CT and MRI have played indispensable roles in initial diagnosis and staging [1]. However, their significance in the field has evolved significantly over time, propelled by the advent of innovative techniques, abbreviated protocols, and novel contrast agents. These continuous advancements strive to enhance image acquisition, refine the diagnostic accuracy of cancer, and optimize patient care. In this comprehensive review, we endeavor to elucidate the current status and recent strides in the application of CT and MRI in PCa imaging, shedding light on their evolving roles and the promising avenues they open for the future of healthcare.

These imaging modalities brought about substantial improvements in diagnosis, treatment, and overall patient care compared to the pre-existing technologies. In prostate cancer's clinical management, CT and MRI have played pivotal roles in initial diagnosis and staging. However, their roles in PCa have evolved significantly over time, driven by the emergence of new technologies. The field continuously explores avenues to enhance image acquisition, refine cancer diagnosis, and optimize patient management, from innovative CT techniques to streamlined MRI protocols and the

introduction of novel contrast agents [1]. Within this manuscript, we endeavor to provide an overview of the present status and recent advancements in CT and MRI for PCa imaging.

2. Method and patient selection

2.1. Patient selection

The total number of patient is 150 including 114 men and 36 women; average age is 64; range of age is between 27 and 91 years old. Patients need to be detected by a clinical 18F-FDG PET/CT examination for staging, restaging, or follow-up of various head and neck cancers. Follow-up scans were performed at least 3 mo after surgery or radiation therapy [2].

There are some limitations about the selection of patient, for example, coma, pacemaker or other contraindication to MRI; symptoms suggestive of subarachnoid hemorrhage (SAH); cannot obtain MRI after 6 hours from final known well time; initiation of thrombolytic therapy, intravenous antithrombotics or anticoagulants, or antithrombotic investigational drug before completion of both imaging studies; or cardiorespiratory instability precluding MRI [3].

2.2. Imaging techniques & processing

Almost all patients check their inner body health by using CT and MRI. All were 5-T scanners installed with echo-planar imaging: Computed tomographic CT scans were performed on one of three fourth-generation scanners: Somatom Plus scanner from Siemens, High Speed scanner both from General Electric. The images were acquired with a thickness of 5 mm throughout the examination following the orbito-meatal plane. Both scanners used an identical pulse slice thickness of 7mm for gradient recalled (GRE) and diffusion-weighted imaging (DWI). The repetition was set to 800 ms at a flip angle of 30° 256 DWI [3]. At UCLA and Suburban Hospital respectively, the field of view was adjusted to be 24 cm and 22 cm for both GRE and DWI sequences; echo time (TE) was 5 ms between at UCLA 6000 ms while those at Sub Hospital from 20 contiguous slices that were interleaved values also between sites with settings at UCLA being 100 ms. In contrast, those at Suburban Hospital, being 72ms since UCLA utilized the Siemens Vision scanner from Siemens Medical System in Iselin NJ. At the same time, Suburban Hospital employed the GE Signa scanner from General Electric Medical Systems [3].

The PET, CT, and MR images obtained were sent to a dedicated review's Advantage Workstation. This workstation allows for the simultaneous examination of PET, CT, and MR images either side by side or in fused/overlay mode (PET/CT; PET/MR imaging). Due to the well-calibrated 3-modality system utilized, there was no need for software-based image registration. A previous study has confirmed image registration accuracy with minimal lateral misalignment (less than 4 mm datasets). This error level is comparable to that determined through phantom measurements [2].

There are two ways, rule-based or pixel classification, to classify the lung regions extraction approaches using Computer-aided diagnosis in chest radiography. Automatic detection of small lung nodules on CT is an old model and provides badly in detection, it utilizes a local density largest algorithm. Connectivity, position features and uniformity were detected in some approaches. There are two strategies of CADs: density-based and model-based approaches. Detection using artificial neural network and fuzzy clustering methods presents two segmentation methods and [4-5, 7] and Early Detection and Prediction of Lung Cancer Survival using Neural Network Classifier have been developed but provide poor detection and identification [6]. Moreover, it uses Curvelet Transform and Neural Network [8], to propose a new technique for LCD identification where curvelet transforms can extract the features of lung cancer CT scan images proficiently.

3. Comparing MRI and CT

The diagnostic strengths of CT and Magnetic Resonance Imaging (MRI) in assessing intracranial hemorrhages. Both modalities effectively visualize primary intraparenchymal hematomas and subarachnoid hemorrhages, with MRI demonstrating particular advantages in detecting microbleeds

and chronic hemorrhages. Both CT and MRI identified intraventricular hemorrhages, although their sensitivity varied. The study found comparable diagnostic accuracy between the two modalities. Notably, imaging within hours of symptom onset underscores the need for rapid and precise diagnoses. In essence, CT and MRI each offer unique insights, and their combined use enhances clinical decision-making in cases of intracranial hemorrhage [9].

Additionally, there are comparative strengths of Magnetic Resonance Imaging (MRI) and Computed Tomography Angiography (CTA) in evaluating aortic aneurysms. MRI, characterized as a robust tool, provides detailed information about aneurysm location, extent, and precise diameter, along with its relationship to aortic branch vessels. However, it emphasizes the importance of obtaining measurements from source images instead of MIP images to prevent an underestimation of aneurysm size. MRI effectively displays mural thrombus but may not reliably detect aortic wall calcifications. On the other hand, CTA emerges as the preferred technique for suspected aortic rupture, boasting speed, widespread availability, and excellent sensitivity and specificity [10]. It identifies key diagnostic features such as active contrast extravasation and high-attenuation hemorrhagic collections, aiding in the swift diagnosis of this critical condition. Additionally, CTA can reveal impending rupture and aortoduodenal fistula, providing valuable insights into potential complications. The choice between MRI and CTA depends on the clinical scenario and the specific diagnostic requirements. While MRI shines in its ability to offer comprehensive anatomical details and lacks ionizing radiation, CTA's speed and accuracy make it indispensable for assessing aortic ruptures. The text underscores that both modalities contribute significantly to accurately diagnosing and managing aortic aneurysms, ensuring patient care is tailored to individual needs [10].

In recent years, the domain of medical imaging has witnessed remarkable technological advancements, notably within Magnetic Resonance Imaging (MRI) and CT. These imaging modalities have evolved into indispensable clinical practice and medical research assets, delivering intricate insights into human anatomy and pathology. Although MRI and CT share the overarching objective of visualizing internal structures, they operate on distinct physical principles, each presenting a set of merits and limitations [13]. MRI harnesses potent magnetic fields and radio waves to craft detailed images, rendering it proficient in soft tissue differentiation and offering exceptional contrast in regions such as the brain, musculoskeletal system, and cardiac structures. Conversely, CT relies on X-rays to generate cross-sectional images, excelling in depicting bony structures and detecting subtle alterations in tissue density. The selection of either modality hinges upon the clinical context and specific diagnostic requisites. MRI enjoys favor for its prowess in soft tissue characterization and its radiation-free nature, while CT's swiftness and precision prove invaluable in scenarios like acute condition assessment and vascular anomaly evaluation. The relentless advancement of MRI and CT technologies holds the promise of further enriching their diagnostic capacities, thereby enhancing patient care and deepening our comprehension of diverse medical conditions [13].

4. Advances of CT and MRI

Starting by illustrating a simplified representation of a CT scanner to explain the assumptions made when deriving the Filtered Back Projection (FBP) algorithm, a conventional analytical reconstruction method. The FBP algorithm assumes certain idealized conditions, such as infinitely small x-ray focal spots, ignoring the shape and size of detector cells, and considering image voxels as points at their centers. These assumptions simplify the mathematical aspects but do not accurately represent the physical reality of CT imaging. In contrast, clinical CT scanners have finite-sized focal spots, non-negligible detector cell dimensions, and actual voxel shapes determined by reconstruction parameters. Additionally, each measurement suffers from photon statistics and electronic noise, introducing fluctuations in the data. These simplifications in FBP can impact the quality of reconstructed images, suggesting that MBIR, which considers these complexities and iteratively refines reconstructions, may offer advantages in terms of image quality and accuracy [11]. In conclusion, micro-computed tomography (μ CT) has emerged as a powerful imaging technique with the ability to provide high-resolution, three-dimensional insights into bone microstructure. This technology represents a

significant advancement in the field of bone research, offering numerous advantages over traditional histomorphometric analysis of thin sections [12]. The potential of μ CT is particularly evident in its application to the study of trabecular bone structure. Researchers have used μ CT to investigate changes in bone architecture in various clinical scenarios, such as osteoporosis treatment trials. In these studies, μ CT revealed intricate details of bone microarchitecture, including parameters like trabecular number, thickness, spacing, and connectivity density. For instance, studies involving osteoporotic women treated with medications like risedronate and strontium ranelate demonstrated the ability of μ CT to capture the subtle yet crucial changes in trabecular and cortical bone microstructure. Such changes, such as shifts from rod-like to plate-like trabecular patterns and alterations in cortical thickness, can significantly affect bone biomechanical competence. The ability of μ CT's to visualize these changes enhances our understanding of treatment outcomes and can potentially help explain reductions in fracture risk associated with certain therapies [12].

The utilization of PET/MRI in oncologic imaging, particularly in the context of prostate cancer, has gained significant traction in recent years. This hybrid imaging modality offers distinct advantages over traditional approaches, such as combining PET with CT. Notably, PET/MRI provides higher resolution for anatomical and tissue details, enhanced sensitivity, and eliminates the exposure to ionizing radiation associated with CT scans. Furthermore, the ability of PET/MRI to simultaneously capture molecular, functional, and anatomical data in a single imaging session streamlines the diagnostic process. As the availability of PET/MRI hybrid scanners continues to expand, its application in prostate cancer management has become increasingly feasible.

A systematic analysis of pertinent studies revealed promising results, with high sensitivity for primary tumor detection and a notable capacity to identify lymph node metastases. Additionally, another meta-analysis confirmed the effectiveness of PET/MRI in detecting primary prostate cancer, with sensitivity and specificity values indicating its potential as a valuable diagnostic tool, particularly in cases of biochemical recurrence [14].

5. Conclusion

In conclusion, the utilization of CT and Magnetic Resonance Imaging (MRI) in the realm of prostate cancer (PCa) imaging represents a dynamic and ever-evolving field. Since their inception, CT and MRI have continually transformed the medical diagnosis and treatment landscape, offering invaluable insights into PCa management. While they have been foundational in initial diagnosis and staging, the contemporary landscape witnesses their roles expanding and adapting to meet the evolving demands of the healthcare ecosystem.

Recent advancements, from cutting-edge CT techniques to streamlined MRI protocols and innovative contrast agents, have propelled these modalities to new heights. These developments are poised to revolutionize the accuracy and precision of PCa diagnosis, further optimizing patient care and outcomes. The remarkable fusion of anatomical and functional data provided by CT and MRI has ushered in a new era of PCa imaging, enabling a holistic understanding of the disease's complexity.

Moreover, the introduction of PET/MRI hybrid scanners has ushered in a new era in oncologic imaging, particularly in the context of PCa. The synergy between PET and MRI offers higher resolution and sensitivity and eliminates the risks associated with ionizing radiation. This novel approach provides a streamlined and comprehensive diagnostic process, exemplifying the potential of technological convergence in modern medicine.

While our exploration of CT and MRI in PCa imaging has illuminated their pivotal roles, it also underscores the need for ongoing research and innovation. The relentless pursuit of enhanced imaging techniques, coupled with a commitment to addressing the unique challenges posed by PCa, will continue to refine and redefine the diagnostic landscape. The potential for earlier and more accurate detection, personalized treatment strategies, and improved patient outcomes remains tantalizing.

In the ever-evolving journey of PCa diagnosis and management, CT and MRI stand as steadfast allies, offering invaluable tools to healthcare practitioners. Their transformative impact on the understanding and treatment of PCa is undeniable, and their future promises even greater contributions

to the field. As we look ahead, we must continue to embrace innovation and collaboration to harness the full potential of CT and MRI in the ongoing battle against prostate cancer.

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Progress in the application of gene editing technology in cancer treatments - Taking CRISPR as an example

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Abstract. The background of CRISPR's development lies in the long-standing need for precise gene editing tools, which culminated in the revolutionary CRISPR-Cas9 system in 2012. This technology has had profound impacts on biology, medicine, and biotechnology, offering versatile applications in gene therapy, agriculture, and research across eukaryotes, prokaryotes, and archaea. In the context of cancer research, CRISPR has shown promise in understanding cancer biology, identifying therapeutic targets, enhancing immunotherapy, and potentially enabling early cancer diagnosis and personalized treatment. However, challenges remain, including the need to enhance precision, address ethical concerns, and conduct rigorous clinical trials. This article examines CRISPR's advancements in cancer treatment and concludes that, when compared to other gene editing methods, it offers benefits like robust targeting, easy operation, and efficient editing. Based on these benefits, the application prospect of CRISPR in enhancing personalized medicine, drug development, and cancer treatment is also discussed in this article. This article highlights the need for addressing precision and safety issues and the importance of interdisciplinary collaboration and stringent regulation in realizing the full potential of CRISPR in cancer treatment. In conclusion, CRISPR offers promising avenues for improving cancer treatment by enabling precise gene editing and personalized therapies. Despite the challenges, continuous advancements in technology and research, along with stricter regulation, will ensure the safe and effective use of CRISPR in cancer, providing hope for cancer patients and shaping the future of oncology research.

Keywords: CRISPR-Cas9, cancer treatments, research progress.

1. Introduction

The background of CRISPR's development stems from the long-term pursuit of gene editing needs, and with continuous exploration and iteration, the CRISPR-Cas9 system was revolutionized in 2012. This application of the natural immune system has led to scientific revolutions in biology, medicine and biotechnology by making gene editing more precise, easy and efficient [1]. CRISPR has a wide range of applications, including but not limited to gene therapy, agriculture, biological research and bioengineering. Over the past few years, CRISPR gene editing tools have found extensive applications across the three major domains of life: eukaryotes, prokaryotes, and archaea. This has proven to be of immense importance in the genetic enhancement of microorganisms and the optimal utilization of microbial resources [1]. At the same time, CRISPR has been used to modify a variety of plant species, such as crop trait improvement [2].

Currently, despite some encouraging advances in cancer research, there are still some important issues and challenges that limit its widespread use in practical applications. The first is the accuracy and specificity of CRISPR, where non-specific cuts or incomplete repairs can occur during editing, leading to unexpected genetic variants or cell mutations. Second, CRISPR editing can raise unpredictable security concerns. Before CRISPR-Cas therapy can reach its clinical potential, it is necessary to overcome possible off-target effects, improve delivery strategies and reduce viral escape [3]. Despite some success in the lab, translating CRISPR into clinical treatment still requires rigorous clinical trials. CRISPR raises a number of ethical questions in cancer treatment, including under what circumstances the technology can be used and how to ensure it is not misused, and developing a clear ethical and legal framework is a major challenge. The use of CRISPR has far-reaching research significance and prospects in cancer treatments.

Studies have found that CRISPR technology can precisely edit cancer-related genes, delve into the biological mechanisms of cancer, find new therapeutic targets, screen out potential drugs, and enhance immunotherapy effects. In addition, CRISPR is also expected to be used for early cancer diagnosis and personalized treatment, providing patients with better treatment options. This paper analyzes the research and application of CRISPR in the field of cancer treatment in recent years, and based on these analyses, evaluates the feasibility and safety of this technology for future application to cancer treatment. These efforts are expected to open up new prospects for cancer research and treatment, providing more hope for cancer patients. An in-depth analysis of CRISPR technology's outcomes in the realm of cancer, when juxtaposed with other technologies, readily reveals its distinct advantages. Its simplicity, efficiency, and excellent specificity have made it a major draw of attention in cancer research.

CRISPR is relatively easy to operate, allowing more researchers to quickly master and carry out a wider range of cancer research. Its efficient gene-editing capabilities allow scientists to accurately modify cancer-associated genes, reducing non-specific effects, leading to a deeper understanding of the mechanisms of cancer development. The specificity of CRISPR allows precise selection of target genes, reducing unnecessary interference and improving the accuracy of experiments. In addition, CRISPR has the potential to overcome the current challenges facing cancer research institutes, provide possibilities for innovative therapeutics, facilitate drug discovery, support personalized treatments, and provide great promise for continuous improvement of cancer treatment. Therefore, the application prospect of CRISPR in cancer is highly anticipated, which is expected to open up new paths for cancer research and treatment.

2. The mechanism of CRISPR-Cas9

The gene-editing tool, CRISPR is based on the CRISPR-Cas system. Its development purpose is to precisely target the modification of the genetic material of organisms. Scientists have engineered various versions of the CRISPR-Cas system, with the CRISPR-Cas9 system being the most prominent. This system employs a specific Cas protein known as Cas9 and a guide RNA fragment to meticulously cleave and alter the DNA of a specific gene. This technique can be used to add, delete or repair genes to make precise modifications to the genome. The CRISPR-Cas system is an acquired immune system that is widely present in prokaryotes and is used to fight foreign genetic elements. This system accounts for about 50 percent of the bacteria and 90 percent of the sequenced genomes of archaea. It comprises two primary elements: the CRISPR array and a set of CAS genes that encode the Cas protein [1]. The fundamental concept of technology combined with the CRISPR-Cas system revolves around editing the genome and controlling the physiological processes in various organisms and cells [4]. The immune system within the context of the CRISPR-Cas system can be categorized into three primary phases: adaptation, processing, and intervention. During the adaptation phase, which is also referred to as spacer acquisition, the system captures new spacers from the invading nucleic material and rapidly incorporates them into the CRISPR array. This process typically involves stable complexes comprising two Cas1 units and a Cas2 unit, sometimes assisted by additional proteins like Cas4 and reverse transcriptase. Moving on to the second phase, the maturation of CRISPR RNA (crRNA) takes place. Initially, the entire CRISPR array is transcribed into precursor crRNA (pre-crRNA) with the guidance of embedded

promoter elements within the guide sequence. Following this transcription, a series of ribonucleases, including Cas nucleases such as Cas6, Cas5d, and Cas12, along with non-Cas nucleases like RNase III and other RNases crucial for maintaining cellular functions (E and PNPase), aid in the conversion of precursor crRNAs into fully functional crRNAs. These mature crRNAs then associate with effector proteins or multi-subunit effector protein complexes to create ribonucleoprotein complexes (RNPs). In the final phase of target interference, RNPs bind to complementary sequences between the target nucleic material and crRNA to recognize and cleave the invading DNA or RNA. This process effectively shields foreign genetic material and accomplishes the interference mechanism [1].

3. Achievements and technical advantages of CRISPR technology

CRISPR has achieved remarkable research results in many important fields and is currently at different stages of development. In biotechnology, CRISPR-Cas technology is used to engineer cell factories that utilize C1 substrates for biomanufacturing, such as reducing greenhouse gas emissions and lowering atmospheric CO₂ concentrations [5]. In the field of agriculture, CRISPR is used to improve crops, improve the quality of crops, mainly focusing on improving the appearance, food quality, fruit substance and nutritional value of crops, improving yield and disease resistance, and some improvements crops have been put on the market [6]. In addition, CRISPR is also widely used in cancer treatment, immunotherapy for infection with viruses, and gene regulation, although some applications are still being researched and developed, CRISPR has made breakthroughs and continues to trigger global scientific research and ethical discussions [7]. Compared with ZEN and TALENs, CRISPR-Cas9 has more advantages. First, CRISPR-Cas9 is more cost-effective. Second, it has higher targeting specificity, easy design of sgRNA, and a variety of feasible editing methods, including vector system modification, direct embryonic gene editing, simultaneous introduction of multiple mutations, and base editing. These indicate the potential safety and success of CRISPR-Cas9 technology in terms of clinical applications [8].

4. CRISPR technology applications in cancer

Researchers have conducted several successful gene-editing studies using the CRISPR-Cas9 system, revealing a number of potential therapeutic targets. For example, by targeting and inhibiting cancer-driver genes, the growth rate of certain tumors has been successfully slowed. CRISPR technology is also being used to repair genetic mutations associated with cancer, providing new prospects for individualized treatment. In immunotherapy, researchers are using CRISPR to improve T-cell therapy and improve the immune system's ability to respond to cancer. Several studies have explored the possibility of using CRISPR for early detection of cancer, providing earlier diagnosis and intervention opportunities by detecting specific DNA markers in body fluids. While further research and clinical validation are still needed, CRISPR brings new promise to the field of cancer treatment and is expected to improve the quality of life and survival chances of cancer patients [9]. However, the development of CRISPR for cancer faces some important problems. First, the precision and specificity of the technique still need to be improved to ensure accurate localization and treatment of tumor cells without harming normal cells. Second, CRISPR raises ethical and safety questions, including concerns about the potential risks that gene editing could bring, and the long-term effects of the treatment. In addition, clinical trials and approval processes will take time to validate the safety and efficacy of CRISPR, which could delay its widespread adoption in cancer treatment. Solving these problems requires interdisciplinary collaboration and in-depth research to ensure that CRISPR is safe and effective in the field of cancer, ultimately helping to improve the quality of life and treatment outcomes of cancer patients [10]. For some table titles and contents, the left side is set to 0.5 mm, mainly to fit the table so that the title is centered. As long as the layout can be reasonable, no indentation can be added.

5. Discussion

In order to solve the problems faced by CRISPR in cancer, researchers have taken a variety of measures. First, they continue to improve CRISPR to improve its precision and specificity [10]. For example,

researchers have developed more precise variants of CRISPR, such as the base editor, for precise single-base repair, reducing unwanted genetic variation. In addition, specific recognition methods targeting tumor cells, such as the use of cancer-specific antibodies or markers to guide CRISPR editing, are expected to reduce the impact on normal cells. Second, basic research plays a key role in addressing ethical and safety issues. Researchers are delving into the effects of gene editing on the organism, including potential adverse consequences, to develop stricter ethical guidelines and regulatory policies [8]. For example, studies have found that certain variants of CRISPR may trigger undesirable cell mutations, raising alarms about the potential risks of treatment. Future trends include better application of CRISPR to personalize cancer treatment, such as by studying a patient's genome to precisely design treatment options. In addition, enhanced interdisciplinary collaboration that integrates basic research with clinical practice can help bring new therapeutic strategies to market faster. In summary, through continuous improvement of technology, deepening research and strict regulation, CRISPR has the potential to improve the future of cancer treatment by providing patients with safer and more effective treatment options in the field of cancer [9].

6. Conclusion

As a revolutionary gene editing tool, CRISPR has achieved remarkable research results in the field of cancer, providing broad prospects for future applications. By precisely editing cancer-related genes, researchers have revealed multiple therapeutic potentials, with some encouraging initial successes in clinical trials. The development of personalized treatment options, tailored to the genetic profile of the patient, will be the trend of the future, providing patients with more effective treatment options. In addition, continued improvements in CRISPR, including more precise editing tools and safer variations of the technology, will help address some of the issues currently faced, such as non-specific editing and ethical issues. To ensure the safe and sustainable use of CRISPR in cancer, interdisciplinary collaboration and stricter supervision are necessary for future research. In short, CRISPR is expected to bring more precise, personalized and effective treatments to the field of cancer, bringing more hope and opportunities to cancer patients. However, in future research, caution should also be exercised regarding the challenges and risks to ensure that widespread adoption of the technology remains centered on patient well-being. In future research, CRISPR will make more breakthroughs in the field of cancer and bring greater hope to the health and survival of cancer patients.

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Cohort analysis for the present mutation of NPM1 in bone marrow cancer

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Abstract. DNA is the most important code for deciphering human genetic information. It finally determines proteins through the central dogma. Therefore, gene mutations can cause various diseases. Cancer is one of the most difficult-to-cure diseases caused by mutations. Acute myeloid leukemia is one of the subtypes of bone marrow cancer, this essay will predominantly focus on the study of the mutation gene NPM1 (nucleophosmin 1). In order to understand the differences between the mutated gene NPM1 and other mutated genes, a cohort study analysis of NPM1 is needed. This research helps better understanding of cancer and the variation of NPM1 compared to the others. By researching and analyzing direct clinical data, this essay will contribute to the understanding of cancer and help turn a scientific blank message into a more simple and understandable manner. However, the limitation of this study is insufficient or even missing data. With the development of research and the improvement of data, future studies will be more accurate and effective, providing a more effective reference for exploring targeted therapy of acute myeloid leukemia.

Keywords: Bone marrow cancer, acute myeloid leukemia, mutation gene NPM1.

1. Introduction

Bone marrow cancer: Bone marrow is the soft, spongy tissue in the center of the bones, this bone marrow contains undifferentiated stem cells, which can develop into different types of blood cells as shown in figure 1, like red blood cells, white blood cells, and platelets [1,2]. The human body tends to produce these cells to replace the old blood cells, therefore they can replicate quickly and mutated blood cells will trigger cancer. There are different subtypes of bone marrow cancer, including multiple myeloma, leukemia, and lymphoma [3]. Different subtypes would be caused by mutations in different blood cells. This study will focus on the mutations of red blood cells, which is commonly known as Leukemia. Inside leukemias, acute leukemias is the type of cancer that grows faster compared to chronic leukemias. To be more specific, this study will focus on the Acute myeloid leukemia (AML) cancer subtypes [4].

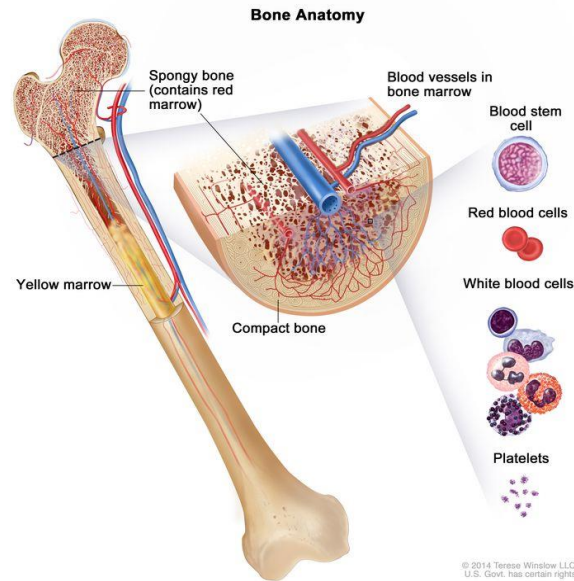


Figure 1. Bone Anatomy [2].

Acute myeloid leukemia: The estimated leukemia in the United States for 2023 is About 20,380 new cases of acute myeloid leukemia and about 11,310 deaths from AML [5]. AML is a disease that targets the elderly and is uncommon before the age of 45. The 5-year relative survival rate for people 20 and older with AML is 28% [6]. In most cases, patients will choose chemotherapy to turn blood cell counts return to within normal limits [7]. On the other hand, stem cell transplant would also be an applicable option for patients. Generally speaking, this is cancer that is based on the development of cancer cells over time, how much is the difference between normal cells and mutated cancer cells This leukemia happens when abnormal leukemia cells produce, it will take over nutrients and rooms from healthy white blood cells, red blood cells, and platelets. Therefore, infection, anemia, or easy bleeding may occur [8].

NPM1 is a protein that encodes a multifunctional phosphoprotein that functions in protein trafficking, pre ribosomal assembly, and ARF- TP53 pathway regulations. Acute promyelocytic leukemia which is a type of AML, is known by a specific translocation between chromosomes 15 and 17, which results in fusion protein promyelocytic leukemia - retinoic acid receptor. This mutation causes loss of nucleolar localization with accumulation of nucleophosmin in the cytoplasm. This is a mutation that occurs in 25% to 35% of cases for adults and 7%- 8% of pediatric cases. Which is a cancer that has an incidence increase over age development [9] (Table 1).

Purpose: The aim of this study is to compare and analyze the clinical case study of patients diagnosed with AML from mutation DPM1 gene will better understand the differences between this specific gene mutation and others, hence that bone marrow cancer has such massive subtypes of cancer, this helps recognize and diagnose cancer better and quicker. There are many available resources or data online provided directly by scientists or researchers, this essay focuses on one specific gene and provides a detailed explanation to fill in the research black of the data. This helps better understanding for the reader and the public.

Table 1. Relationship between gamma radiation dose and myocardial damage [3].

Dose	n	ECG or cTnI abnormalities	Myocardial injury rate(%)
<30Gy	26	0	0
30~50Gy	16	2	12.5
50~60Gy	12	2	16.7
>60Gy	10	4	40

2. Methodology

In order to get clinical data from patients, this study will be using the Genomic Data Commons Data Portal (CGD data portal) [10], This website provides cases of different patients who have been diagnosed with cancer. Which also provides data analysis functions.

So, in order to build a cohort study for NPM1, first research and limit the cases under Bone marrow cancer, acute myeloid leukemia, NPM1 gene. As shown in figure 2, this creates data sets that contain 47 mutated NPM1 cases in one data set (S1), The other hand creates another data set (S2) which is the collection of all the bone marrow, AML cancer but without NPM1 mutation cases, which has 4261 cases in total. Both of the data will have no cases that share the same gene mutations. In order to present clear contrast and demonstrations. After creating the 2 sets of data, plug them into the cohort comparison and the data will show the following analysis of different criteria: survival rate, sex, age at diagnosis, and vital status. These criteria indicate the full range of basic variables that contribute to the prevalence of cancer.

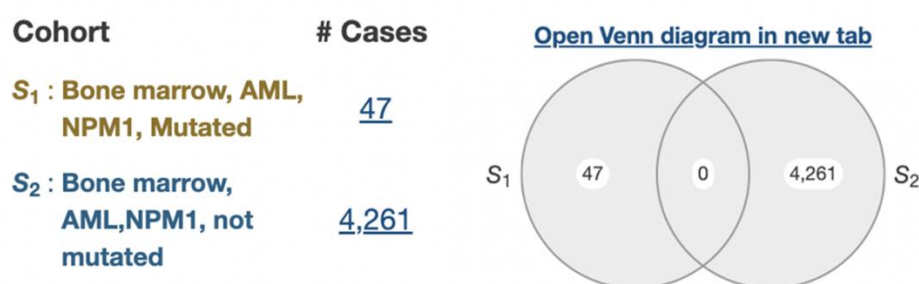


Figure 2. GDC data portal cohort data sets.

3. Results

3.1. Survival rate

The survival rate for both data sets across 8 years is shown in figure 3. Both data sets present an overall trend of decrease in the survival rate. Both data sets have a sharp decrease in survival rate from the first 2 years of diagnosis, which decreased from 1.0 to ~ 0.3 survival rate. Hence the limited case for the mutated NPM1 data set, the trend for S1 stops from year 2. Then S1's survival rate slowly stagnated after year 5, which almost presents a near 0.0 survival rate. Therefore the overall survival rate for S1 is 26% and 21% of the survival rate for S2.

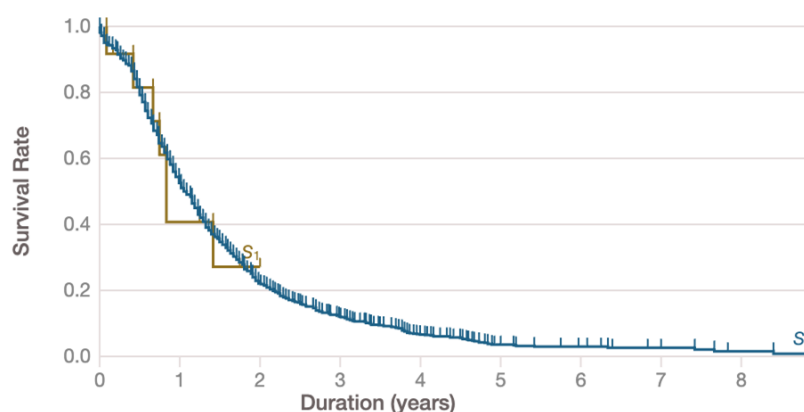


Figure 3. Survival rate of S1 and S2.

The overall survival rate plots communicate certain information, this might indicate that there is not much difference between the incidence rate from the mutated NPM1 and others, As long as it is AML

the survival rate for this cancer will be shown in the high incidence rate and most of them would not be able to survive longer than 5 years. The overall survival rate would be around 20-30%. Therefore there is not much difference between the incidence rate of AML cancer.

3.2. Gender

Figure 4 shows the percentage of cases contribution from males and females. Based on the observatory, there is an overall decrease or less percentage of cases for females compared to males, no matter data sets S1 or S2. In the S1 data set, there is 59.57% of male and 40% of female, in the S2 data set, there is 53.81% of male group and 45.04% of female group.

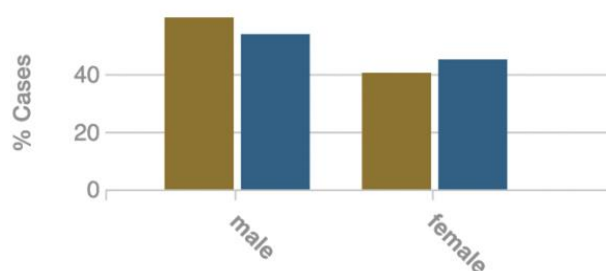


Figure 4. Sex distributions.

Therefore, it also indicates that the prevalence of cancer is generally similar from the two data sets, which shows there is no big variation between genes with NPM1 mutations and other gene mutations. But this chart shows the possible target group of people, which is a male community, and the percentage of cases for both datasets, both have around 10% more cases in males than females. This draws the conclusion that males might have a higher risk of having AML cancer.

3.3. Age at diagnosis

Figure 5 presents the percentage of cases across different ages. In this criteria, the difference between the two data sets is becoming obvious, Data set 2 without the NPM1 mutation gene, most of the percentage of cases around the age of 0 to 20 years old, on the other hand, on S1, most of the percentage of diagnosis is around of the age between 40 to 70 years old.

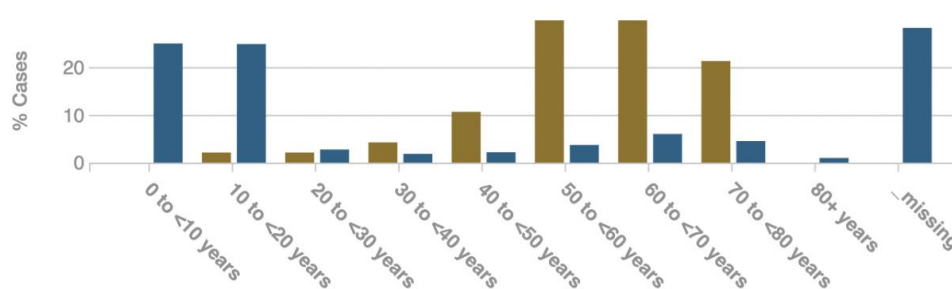


Figure 5. Age at diagnosis for S1 & S2.

From these observations, it is likely to understand the difference between the NPM1 gene and other mutated genes. In the target community of S2 which is another mutation gene, children are at higher risk of being diagnosed with AML that contain other mutated gene, but for S1, which is NPM1, it is more specific down to the community of age between 40-70 years old populations, therefore adult and middle age people are in a higher risk of getting AML with NPM1 mutation gene.

3.4. Vital status

Figure 6 shows the vital status of patients who are diagnosed with AML cancer. However, there are variations between the group with NPM1 mutations and without NPM1 mutations. In S1, There is a higher percentage of cases shown dead than alive, which is 55.32% and 36.17% respectively. But, there is a higher percentage of alive for S2 than dead, which shows 39.99% and 31.47% respectively.

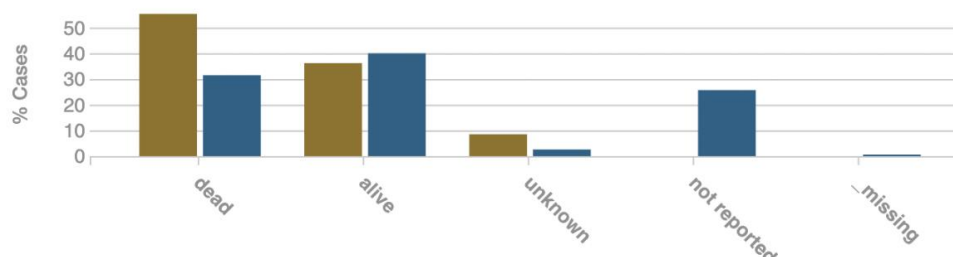


Figure 6. Vital status for S1&S2.

Therefore, It is shown that the variation between the NPM1 gene and others. People who are diagnosed with NPM1 would risk a higher and lesser possibility of survival, but people who are diagnosed with other gene mutations might have a higher rate of cure or survival. This indicates the level of development of the cure method for both gene mutation and the incidence rate for different gene mutations.

4. Discussion

Based on the observations of different categories, the general pattern is shown. In the survival rate, both data sets show a sharp decrease in survival rate access times. For the target community, the chart shows, that in both data sets, males are at higher risk of AML compared to females. For age at diagnosis, normal AML tends to have patients spread around the age of 0 to 20 and middle ages. However, NPM1 showed a greater proportion of elderly in the target community. For vital status, NPM1 mutations tend to have more death compared to others.

5. Conclusion

In conclusion, combine the observation pattern and background knowledge. Acute myeloid leukemia is a cancer that has a high mortality rate. Therefore this is a serious and crude disease. The elderly and male populations should pay more attention to cancer because they have a higher prevalence of diseases. On the other hand, inside AML elderly and middle-aged people tend to become the high prevalence populations, but particularly NPM1 elderly are more affected by this mutation. This pattern might help with quicker diagnosis of the diseases. Lastly, NPM1 mutations show a higher mortality rate compared to other AML mutations, this might indicate the detrimental effect of the cancer. This might draw more attention to these specific mutation genes and develop more treatments or preventions.

Therefore, this observed data and conclusions could be used for further analysis and purpose for the research of NPM1 gene mutations, Proper and reliable observatory experiments would help increase the change of lab experiments and decrease the risk of failure. For this research study, there are also many limitations, like the cases from the CGD data portal are not enough to represent the entire NPM1 cancer research, and only 47 cases would not be enough and viable for the research. Also, there is not enough data for all the criteria, for instance, the NPM1 survival rate plot is not enough which decreases the reliability of the graph and hypothesis. In different charts, there are many “not reported” “missing” and “unknown” data. This is also a limitation of data, which is not accurate and even missing. There are also very big limitations for clinical data analysis, the maintenance of valid and reliable data from patients, the ethical problem of security and protections, and the problem of getting large amounts of data would all be considered as the limitations of this research.

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Potential application of *Saccharina japonica* and its extracts in cosmetology

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Abstract. *Saccharina japonica* (*S. japonica*), a type of brown algae widely consumed in east Asia due to its enormous health benefits, has now been greeted with great expectation in the field of cosmetics. Although many cosmetological functions of *S. japonica* extracts have been reported, there is still a lack of comprehensive review on the potential application of *S. japonica* and its extract in cosmetology. This article aims to sum up the cosmetological functions of *S. japonica* extracts, including anti-inflammation, skin whitening, anti-oxidation, and anti-bacterial activities. Moreover, the production of bioactive chemicals from *S. japonica* is explored, hoping to provide new insights into cosmetic manufacturing. Study exploration was conducted using online databases (PubMed) using the keywords *Saccharina japonica*, *Laminaria japonica*, kelp, cosmetics, anti-inflammation, skin whitening, anti-oxidation, anti-bacterial, etc., focusing on established pre-clinical trials proving relative bioactivities.

Keywords: *S. japonica*, cosmetic, anti-inflammation, skin whitening, anti-oxidation

1. Introduction

Recent years witnessed a sharp rise in the research of bioactive compounds derived from algae. Apart from being used as food, cosmetic preservatives and antioxidant agents, several health advantages have also been reported [1]. *Saccharina japonica* (*S. japonica*), formerly known as *Laminaria japonica* (*L. japonica*) before 2006, is a familiar brown algae mainly used in food industry given its rich polysaccharides, iodine, vitamins, mannitol, mineral elements and other functional substances [2]. *S. japonica* is more often referred to as “kelp” in English. It is most widely dispersed and economically grown in various temperate coastal regions, with significant yields in east Asian nations including China, South Korea, and Japan. As was reported, about 4.8 million tons dehydrated *L. japonica* can be reaped yearly along the coast of China [3].

Due to rising consumer demand for natural ingredients, substances with marine and maritime origins are being used more frequently in skin care products. *S. japonica*, almost the most important marine commercial crop, is anticipated to play a crucial role in future cosmetology. However, there is still a lack in comprehensive reviews of how *S. japonica* and its functional chemicals can be utilized and commercialized in the cosmetology industry. This review aims to sum up the potential functions of *S. japonica* extracts and chemicals purified from *S. japonica* that can provide insights in developing novel and natural cosmetic products.

2. Anti-inflammation activity

First, we mainly focus on the anti-inflammatory activity of chemicals derive from *Saccharina japonica*.

Found in brown algae and echinoderm, fucoidan belongs to sulfated polysaccharide and contains high ratios of L-fucose and a fraction of other types of sugars like galactose, glucuronic acids, mannose and xylose [4]. Former research has shown that fucoidan exhibits a variety of bioactivities, including antioxidant, anticoagulant, and bacterial homeostasis, with the bioactive effects mostly determined by the its molecular weight(MW) [5].

Ni et al. tested the physicochemical properties of LJSF4, a fucoidan purified from *Saccharina j.*. They measured its anti-inflammatory activity and study the molecular mechanisms behind, with both in vitro and in vivo models tested for the first time. It is well wroth mentioning that zebrafish model was opted for in vivo tests, which is generally regarded as the most suitable model for effective measurement of anti-inflammatory activity [6]. They found that when conducted on RAW 264.7 cells for the invitro test, LJSF4 exhibited excellent anti-inflammatory activity, where reduced the production of pro-inflammatory cytokines (TNF-, IL-1, and IL-6) and nitric oxide (NO) was noticed in a dose-dependent way. Also, by lowering cell death and the formation of ROS and NO, LJSF4 had protective effects in zebrafish under exposure to LPS. When it comes to practical production in cosmetic industries, future development and application of LJSF4 could be facilitated thanks to its comparatively simple structure. Moreover, *S. japonica* is broadly cultured with abundant production and industrial supply. These two factors together make it feasible for massive production of *S. japonica* fucoidan, a highly potential anti-inflammatory chemical expected to be applied in the functional food and cosmetic industries [5].

3. Skin-whitening effect

The skin-whitening effect is also what people want to include in an ideal cosmetic. To deal with darkened skin and dark spots on the skin, Tyrosinase (TYR), which is the core rate limiting enzyme in melanogenesis, is usually targeted for inhibition to reduce melanin production and treat skin hyperpigmentation [7]. Due to the mild reaction conditions and preservation of sulfate radicals, it has already been a established method to degrade fucoidan with the help of fucoidanase derived from *Flavobacterium RC2-3*, a fucoidan-degrading bacterium [8]. Using such purified fucoidanase, Chen et al. found that in the MW range above 5 kDa, the smaller MW of fucoidan tended to exhibit enhanced whitening activity. When it comes to fucoidan of molecular weight within the range of 5-10 kDa, highest tyrosine inhibitory activity as well as antioxidant activity and prominent anti-melanogenesis ability were verified in B16 cell models [9]. This study fundamentally revealed the relationship between MW and whitening activity of fucoidan, providing an insight for further transforming fucoidan into a new applicational chemical in skin-whitening products.

4. Anti-oxidation activity

Oxidative damage is also what draws people's concerns in cosmetology, because it has a significant part in skin aging process, both intrinsically and even more so extrinsically. Meanwhile, in the skin, reactive oxygen species are responsible for the aging process to a greater extent than they are in any other organ [10].

A balance between oxidants and antioxidants in the body can be maintained by increasing dietary antioxidant intake or consumption of foods rich in antioxidants [11]. Phlorotannins are a group of major polyphenol secondary metabolites found only in brown algae, including *S. japonica* [12]. Phlorotannins have a wide range of bioactivities and health advantages, including antibacterial, anti-inflammatory, antioxidant, antidiabetic, anti-HIV, and anticancer properties. Bai et al. found that the phlorotannins encapsulated by polyvinylpyrrolidone nanoparticles (PPNPS) showed a slow and steady kinetic release of phlorotannin in simulated gastrointestinal fluid. Also, the encapsulated phlorotannins were proved non-cytotoxic in HaCaT keratinocytes and could diminish the production of endogenous reactive oxygen species (ROS). Because of this, PPNPS may provide an effective platform for the application of phlorotannin in the pharmaceutical and cosmetics fields [13].

5. Anti-bacterial effect

When it comes to anti-bacterial effects, the *L. japonica* essential oil (LJEO), an inexpensive but favorable resource extracted by microwave-hydrodistillation, is reported to manifest strong antibacterial activity. Free radical scavenging and antioxidant activity were also discovered in the oil, making it promising for application in the food, cosmetics, and pharmaceutical industries [14].

Extracts of *S. japonica* and *Rosmarinus officinalis* also display antimicrobial effects against oral pathogens [15]. When one of the two extracts were applied jointly with other anti-microbial chemicals like chlorhexidine digluconate or Protamine sulfate, enhanced anti-bacterial effect could be attained at certain concentrations of both counterparts compared to their respective activities [16]. Hameury et al. saw the lack in ex vivo tests, so they performed a thorough assessment of the activity on the skin of combined ingredients from marine and maritime origins. Using label-free quantitative proteomic analysis, the clinical benefits was predicted when applied in a skin care product. Compared to untreated control groups, 64 proteins were evidently regulated after treatment. Assisted with computer data processing, they found that in epidermis and dermis tissues, these evidently regulated proteins are involved in gene expression, cell survival and metabolism, inflammatory processes, dermal extracellular matrix synthesis, melanogenesis and keratinocyte proliferation, migration, and differentiation. Their results suggested that the ingredients tested could help maintain epidermis and dermis health, expected to minimize the noticeable signs of skin aging [17].

6. Enhancement of kelp polysaccharide extraction process

After talking about the potential applications of *S. japonica* in cosmetology, how the producers are going to extract and purify these functional ingredients but still preserves their activity may be rather significant and difficult, which are likely to require loads of trials. Currently, hot-water, alkline solution, acidic solution, enzymatic and microwave extraction have been used in kelp polysaccharide extraction. As may be an inspiration, Statistics-based response surface methodology (RSM) was adopted by Yu & Chao to magnify the productivity of kelp polysaccharide extraction process. They found out that the optimal conditions were as follows: pH 3.4, temperature 83°C, extraction time 3.95 h and ratio of water to kelp 1:23. Under the above conditions, the production of kelp polysaccharide was 1.26%, with high scavenging percentages of free radicals. Also, an increase in the biosynthetic activity of collagen can be realized at certain kelp polysaccharide concentration. The results above pointed out that kelp polysaccharide is expected to make a good candidate for future use in cosmetics given its high effectivity plus relatively easy production [18]. However, these factors require further verification before being applied in real production due to an amplification in industrial extraction and purification systems.7. Insights in kelp blanching water polysaccharides

Nowadays, seaweeds contribute to half of all mariculture worldwide and are now a major participant in bio-based businesses [19, 20]. Hot water blanching, which is often the first step in the seaweed processing, reduces postharvest quality decline but also produces a significant amount of hydrothermal waste. Notwithstanding the recent realizations that it is likely to contain beneficial chemicals, the blanching waste is still largely untapped for bioactive molecules [21, 22].

Using *Saccharina j.* blanching water as example, 2.9 g/L of polymeric substances were efficiently isolated by ultrafiltration, implying biopolymer coproduction potential of ~5.8 kt from blanching wastewater of current kelp industry. After purified by anion exchange chromatography, the remainings of blanching water polymers mainly consisted of acidic polysaccharide. The biopolymers manifested different yet overlapping structural features with polysaccharides extracted by hot water, in possession of high levels of fucose, uronic acids and sulfate. Additionally, the polysaccharides were proved to be compatible with cosmetic creams, adding to the cohesion and freshness, which suggested the potential cosmetic values for such natural biopolymers from the seaweed blanching water untapped [23]. This deserves further research and development to discover practical strategies and mature techniques to reutilize the valuable ingredients in the kelp blanching water, which used to be treated as wastes.

7. Conclusion

From what is introduced above, *S. japonica* extracts exhibit ideal cosmetic effects like anti-inflammation, skin-whitening, antioxidation, and anti-bacteria, with all these proven activities supported by pre-clinical experiments. What's more, when used in combination, these bioactive components purified from *S. japonica* are likely to provide multiple efficacies or even new unexpected effects.

However, before being commercialized and industrialized, these chemicals still require ample verifications, like clinical trials, which are of the utmost significance and most time-consuming. The final dose applied to cosmetic products should be carefully determined by weighing both safety and efficacy. Moreover, when it comes to the research and development stage of cosmetic products, the preservation of the bioactivities of functional components deserves fine experiments to optimize the effectiveness of *S. japonica* extracts. The form of how manufacturers make their cosmetic products can no longer be limited to applicational products like face cream and lotion; oral preparation and injection may well be taken into consideration, with the phlorotannin nanoparticle mentioned above as a good example.

To answer the call for resource saving and sustainable development, during the processing of raw kelps, it is desirable that the efficiency of extraction be elevated while the energy consumed and the waste produced be lowered as much as possible. Apart from the increasing demand for technology development, reconsidering where the raw materials come from may provide new insights, with kelp blanching water—an initial industrial waste—as a possible new source of raw materials in cosmetics.

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Antiviral research and detecting viral nucleic acids based on the CRISPR-Cas technology

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Abstract. Viruses constructed from nucleic acids, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), are encased in a protein covering and require a living cell to reproduce. Symptoms of viral infections can range from asymptomatic to severe illness, and their impact has become increasingly apparent in recent years. For instance, globally, the Corona Virus Disease 2019 (COVID-19) epidemic alone has resulted in millions of infections and millions of fatalities. Consequently, the development of effective tools for antiviral resistance is an urgent need. Early detection of viral infections can be achieved through nucleic acid detection, while gene editing techniques offer a promising solution for treating and counteracting the harmful effects of viruses, thereby minimizing economic and property loss. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated (CRISPR-associated, Cas) protein system (CRISPR-Cas) is an emerging gene editing tool that has demonstrated remarkable accuracy and efficiency with its rapidly expanding applications. This review delves into the principles and applications of Cas12a, Cas13, and other systems in nucleic acid detection, highlighting the significant contributions of Cas9, Cas12a, Cas13, and other systems in establishing resistance against DNA and/or RNA viral infections, and outlines the strengths and limitations of the CRISPR-Cas system in current applications. Precautions and suggestions for the system's safe application are also presented, with an optimistic outlook for its prospects.

Keywords: CRISPR-Cas, virus, viral nucleic acid detection.

1. Introduction

Viruses are small, uncomplicated organisms that lack cells and have a single kind of nucleic acid, either RNA or DNA. They rely on living cells to parasitize and reproduce through replication, making them unique infectious agents. A virus lacks its enzymatic system and metabolic apparatus, being composed only of a strand of nucleic acids and a protein casing. Therefore, a virus can only replicate, transcribe, and translate within a host cell. Once inside the host cell, the virus can use its resources to complete its life cycle and create a new generation of viruses based on the genetic information found in its nucleic acid. While some viruses, such as phages and insect viruses, can be beneficial for treating bacterial infections and agricultural pests, respectively, many viruses can be harmful to human life, such as Human Immunodeficiency Virus (HIV) and rabies. Additionally, viruses such as influenza and hepatitis viruses can cause disease, while others like the Potato Virus Y (PVY) can damage property.

Antiviral drugs are a class of medication used to treat viral infections, such as interferons and antibodies. Interferons are man-made replicas of natural interferons that can slow or halt viral replication, although they may have side effects [1]. Antibodies are primarily found in the blood of people who have recovered from viral infections (recovery serum) or in the laboratory from altered living cells that produce targeted antibodies (monoclonal antibodies). Unfortunately, many viral infections do not have effective antiviral treatments available. The way antiviral drugs work is by disrupting viral replication. However, viruses are very small and replicate inside cells, using the cell's metabolic functions. Because of this, antiviral drugs are only able to target a limited number of metabolic functions within the cell.

In contrast, bacteria are larger and typically replicate outside of cells, allowing antibiotics to target many different metabolic functions. This makes developing antiviral drugs more challenging than developing antibiotics. Additionally, while most antiviral medications are only effective against one or a few viruses, antibiotics are frequently effective against a wide variety of bacteria. Furthermore, antiviral drugs can have toxic effects on human cells, and viruses can become resistant to them.

To improve the effectiveness of viral infection treatments and minimize damage, scientists are exploring genetic engineering to detect viruses and advance prevention and treatment. The CRISPR-Cas methodology is frequently employed by bacteria to be a defense mechanism against foreign DNA incursions. This system employs short palindromic repeats, which use small RNAs to detect and neutralize invading DNA with specificity. There are two classes in the system, one for types I through IV (excluding II) and one for all other types. The system's specificity not only grants it powerful gene editing capabilities but also permits very sensitive identification of certain DNA sequences. As a result, Utilizing the CRISPR-Cas methodology, viral DNA may be found rapidly and selectively with high sensitivity. It can also be genetically engineered to create organisms that are resistant to DNA infection in plants and animals, making it effective against exogenous DNA. It's extremely probable that the unique and cutting-edge genome engineering tool, the CRISPR-Cas methodology, will make a significant contribution to the identification, avoidance, and management of viruses.

This review seeks a thorough rundown of the CRISPR-Cas system as well as its potential in human anti-virus efforts. It delves into the system's working principle, and application scenarios, as well as its pros and cons. By doing so, this review focused on shedding illumination on the development direction of the system, offering valuable insights and ideas for future researchers.

2. Mechanism of action of CRISPR-Cas

CRISPR is a short palindromic repetitive sequence found only in prokaryotic cells, i.e., bacteria and archaea, and genes associated with it are known as CRISPR-associated genes (cas genes). Three separate mechanistic steps make up the CRISPR-Cas immunization process: adaptation, expression, and interference.

During the first phase, bacteria integrate new spacer regions from viral genes upon phage attack, and the bacterial cellular resistance against bacterial infections is changed by the insertion or deletion of certain particular spacer regions [2]. Therefore, microbes have phage resistance due to the combination of CRISPR and Cas genes, and the specificity of the resistance depends on the similarity of the spacer-phage sequences. Cas proteins are also involved in the adaptive phase of prokaryotic cells, and the most conserved of them is the Cas1 protein [3]. Cas1 proteins with integrase activity are usually grouped in a synthetic complex with Cas2 proteins with endonuclease activity, which together catalyze the introduction of spacer regions [2]. A new spacer region is created when Cas1 enzymes cut a portion of an alien species' genome close to the Proto-spacer Adjacent Motif (PAM) and integrate it into the CRISPR locus. During this process, CRISPR RNA (crRNA) undergoes transcription and changes to become mature RNA [4]. In prokaryotic cells, the CRISPR motif is the region that preserves infected foreign genetic information.

During the expression phase, a DNA strand with a CRISPR site is transcribed into a messenger RNA (mRNA) called pre-crRNA. Numerous repeating CRISPR complementary sequences and alien species' genomic sequences can be found in pre-crRNA [3]. crRNAs are formed from pre-crRNAs, and their final compositions vary for different Cas types, which are classified into I, II, and III types. A repeated

CRISPR genome and a foreign species' genome are present in the crRNA of process type I. The foreign genome sequence repeats do not create loops with repetition, but the CRISPR sequence does, and as a result, the crRNA is digested by the Cas6e and Cas6f nucleases. The trans-activating CRISPR RNA (tracrRNA) molecule plays a crucial role in process category II. Process type III involves the direct destruction of both the foreign species' genomic sequence and every repeat of the CRISPR sequence by the Cas6 nuclease [4].

During the interference phase, specially encoded crRNAs are integrated into Cas proteins as guide RNAs to form CRISPR-Cas complexes [4]. This complex uses information from the exogenous genome in the crRNA to recognize, detect, and inactivate it. As a result, the CRISPR-Cas methodology is commonly utilized within agriculture as well as industry to provide virus resistance to cash crops [5]. In addition, the system is a potent tool for genome research, allowing the editing of gene sequences in a variety of species using different vectors.

3. Utilizing the CRISPR-Cas system to identify viral nucleic acids

The increasing frequency of epidemics as well as attacks by disease-causing viruses has led to a growing need for quick, precise, and inexpensive nucleic acid detection techniques. Studies have demonstrated that DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) [6], Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) [7], one-Hour Low-cost Multipurpose highly Efficient System (HOLMES) [8], and other assays may become excellent biomedical diagnostic tools.

3.1. DETECTR

The RNA-guided CRISPR-Cas12a (Cpf1) proteins are an integral component of the bacterial adaptive immune system, binding and cleaving DNA. Similar to CRISPR-Cas9, in genome editing, Cas12a appears to be used similarly due to the capacity resulting in specific breaks in double-stranded DNA (dsDNA). To be more precise, RNA-directed DNA binding triggers Cas12a's non-specific single-stranded DNA (ssDNA) cleavage function, which breaks down ssDNA molecules. In contrast, other Cas12 enzymes share this common feature of target-activated non-specific single-stranded deoxyribonuclease (ssDNase) cleavage [6].

DETECTR is a technique that Chen et al. developed by combining Cas12a-ssDNAase activation with isothermal amplification, which has the same sensitivity for DNA detection as for human papillomavirus (HPV) and other common viruses [6]. Amidst the COVID-19 pandemic, Broughton et al. utilized a method called loop-mediated amplification (RT-LAMP) to simultaneously amplify and transcribe RNA from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM) [9]. After this, particular coronavirus sequences were found using Cas12, and reporter molecules were then cleaved to confirm the presence of the virus [9]. The detection process utilized only a few necessary primers, detection reagents, and instruments [9]. It is evident that DETECTR currently serves an intriguing purpose in discovering epidemic infections quickly and effectively.

3.2. SHERLOCK

The RNA-directed ribonuclease (RNase) Cas13, which consists of four different family members (Cas13a-d), generates several cleavage locations in RNA targets' single-stranded regions based on particular base preferences. Additionally, it exhibits promiscuous RNase activity that is dependent on the target and causes bystander RNA molecules to be trans-cleaved, an effect that is referred to as side branching activity. Hence, the detection of nucleic acids with side branching activity can be achieved by using reporter molecules that are fluorescently labeled, antigenically labeled, etc., along with steps such as preamplification and the T7 RNA polymerase promoter introduction, i.e., specific and highly sensitive enzyme-mediated unlocking (SHERLOCK) [7].

Using the Cas13a-based molecular detection platform SHERLOCK, particular variants of the Zika viruses, dangerous microorganisms, human DNA genotyping, and changes in cell-free cancer DNA may all be detected [10]. Additionally, SHERLOCK reaction materials are easily reconstituted on paper for usage in the field after being stored for long-term preservation without a cold chain [10].

3.3. HOLMES

SHERLOCK is very convenient for detecting target RNA, but since in vitro conversion from DNA to RNA needs to be performed prior to the experiment, it can be time-consuming to identify the sequences of DNA. This led to the development of the HOLMES.

Cas12a of the type 2 V-A system performs side branching cleavage of non-target ssDNA when forming a ternary combination of target DNA, crRNA, and cas12a [8]. Taking advantage of this property, HOLMES uses a bursting fluorescent ssDNA reporter as a probe and together alongside the Cas12a/crRNA twofold multifaceted DNA target forms a ternary configuration if it has been included within the response environment, which then trans-cut the non-targeted single-stranded DNA reporter, allowing the fluorophore to separate from the bursting agent and emit Hexachloro (HEX) fluorescein [8].

In 2019, a group of researchers from the Chinese Academy of Sciences and others [11] developed HOLMESv2. They developed it by using a V-B-type mechanism and thermophilic RNA-guided Cas12b. By merging target identification and nucleic acid enhancement into a single procedure, they also made the operational procedures simpler. This was done to prevent cross-contamination.

With this method, human single nucleotide polymorphisms (SNPs), DNA viruses, RNA viruses, etc. can be detected [8, 11]. In HOLMES and HOLMESv2, the nucleic acid may be detected quickly and cheaply by using PCR, which uses inexpensive chemicals instead.

3.4. Other detection methods

When utilizing Casase without target upstream pre-amplification, the majority of CRISPR-based diagnostics report a limit of detection (LOD) in the picomolar range [12]. This LOD only permits identification when the sample DNA or RNA concentration is sufficiently high, as is the case for COVID-19 at high concentrations early in the infection. Therefore, to increase the sensitivity, LwaCas13a can be used in conjunction with Csm6, a CRISPR III RNase. Since LwaCas13a has side branch cleavage activity, Cas13 can produce a Csm6 activator, which enables amplified signal detection [13].

Furthermore, methods such as pre-amplification and quantification of biomarker concentrations can be used to obtain the appropriate LOD for clinical utility.

4. The implementation associated with CRISPR-Cas platforms in addition to antiviral tactics

The CRISPR-Cas system, a mechanism exclusive to prokaryotes, has made a significant contribution to the protection of prokaryotes against external genetic material. As a result, people associate the system with the introduction of eukaryotes imparting immunity against relevant viruses, or with the employment of the system to directly or indirectly attack viruses in order to cure viral illnesses that are infectious. Most recently, with the continuous maturation of related technologies, this system has gradually become an antiviral tool with vast prospects, bringing gospel to the fight against viruses.

4.1. Establishment of virus resistance

4.1.1. Establishment of DNA virus resistance. Depending on the dependence of the virus on the host, the genome of influenza viruses may be targeted for destruction using the CRISPR-Cas methodology, and the use of this methodology can target the destruction of host factors to resist viral infection and inhibit viral life activities as well.

The type of CRISPR-Cas methodology that has been studied more extensively is type II. Two tiny RNAs, crRNA as well as tracrRNA, and a single guide RNA (sgRNA), a form of these two small RNAs that have been artificially combined, are employed to lead the Cas9 effector DNA endonuclease to DNA targets in this system [14]. The 20-base pair seed sequence and the 3-base pair PAM (5'-NGG-3') make up a common targeting location for the CRISPR-Cas9 methodology following the Watson and Crick requirements are met, that is, Cas9-mediated dsDNA breaks occur when the CRISPR-Cas9 target, as well as the Cas9-bound sgRNA, complement each other. The host DNA repair mechanism as well as

one of the non-homologous end joining (NHEJ) processes often resolve this [14]. Random nucleotide insertions, deletions, and substitutions around DNA cleavage sites take place during the reparation of DNA caused by NHEJ. As a result, the viral genomic critical regions that code for proteins and/or cis-regulatory components are disrupted, which stops the virus from replicating and eventually causes the host cell to become antiviral.

Based on the principle of action of the type II system, Kennedy et al. [15] targeted and disrupted the E6 and E7 genes in SiHa and HeLa cells, fruitfully blocking their expression and accelerating apoptosis of infected cells, which in turn prevented virus transmission.

4.1.2. Establishment of RNA virus resistance. As the main types of plant viruses, for RNA viruses, Cas13a or FnCas9 is able to strike the genome of the virus, or Cas9 could be employed for targeting the host factors of RNA viruses.

By hindering the ssRNA phage's ability to be cleaved by MS2 in *E. coli*, Cas13a was shown to provide immunity to phage; in vivo and in vitro, it breaks down the intended ssRNAs with the direction of the control of a crRNA containing a 28-nucleotide spacer region sequence [16]. Zhang et al. [5] reprogrammed sgRNA-FnCas9 to produce cucumber mosaic virus (CMV)-resistant plants, also by *Agrobacterium*-mediated transformation.

Alternatively, targeting viral host factors using Cas9 is also a way to establish viral resistance. The eIF4E genome in cucumbers has been selected for editing using CRISPR-Cas9 by Chandrasekaran et al. [17], and they screened pure non-transgenic cucumbers with a knockout of the eIF4E gene, which had significant viral resistance through backcrosses.

4.2. Treatment of viral infections

Since the Hepatitis B Virus (HBV) represents a DNA virus, when it reaches the nucleus, its genome is covalently closed circular DNA (cccDNA). Reverse transcriptase inhibitors, such as nucleic acids and nucleotide analogs, are the mainstay of current HBV therapy. Lin et al. [18] designed eight gRNAs against type A HBV, which together with Cas9 protein significantly reduced HBV core antibody and the surface antibody to HBV in Huh7 cells containing HBV expression vectors, indicating that the CRISPR/Cas system can cut HBV-specific sequences in cell models. Subsequently, researchers further demonstrated in a mouse model, in which hepatocytes plasmid carrying the HBV nucleotide might be destroyed by CRISPR/Cas9, thereby removing the plasmid and leading to a decrease in the level of HBV surface antibody within the blood.

Herpes viruses are a class of large DNA viruses with an envelope, which are best characterized by a latent cycle that allows them to under-express their proteins in cells thereby evading immune surveillance, and ultimately leading to lifelong infection because the host cell is unable to remove the virus from the infected cell. Herpes Simplex Virus type 1 (HSV-1) is one of the human neuroleptic α -herpesviruses, and Roehm et al. [19] effectively inhibited HSV-1 replication and reversed HSV-1-induced disassembly of pro-granulocytic leukemia nucleosomes through CRISPR-Cas9-based ICP0 targeted disruption.

Cervical cancer in women is brought on by high-risk Human Papillomavirus (HPV) infection, and although various prophylactic vaccines against HPV infection have been successfully developed, specific antiviral drugs against HPV are not yet available. Kennedy et al. [15] targeted and disrupted the E6 as well as E7 genes in SiHa et HeLa cells with HPV infection, accelerated apoptosis of infected cells, and then cleared HPV to block its transmission.

4.2.1. Treatment of RNA virus infections. The primary pathogen responsible for Acquired Immune Deficiency Syndrome (AIDS) is HIV Type-1 (HIV-1), and although the advent of highly active antiretroviral therapy (HAART) has changed AIDS from a deadly and untreatable to treatable and manageable, its lifelong persistence and incurability have become a huge burden for many AIDS patients. The drawback of HAART's inability to cure AIDS lies in its inability to remove HIV DNA integrated into chromosomes, and thus high hopes have been placed on the CRISPR-Cas9 method for eradicating

the HIV genome. The CRISPR-Cas9 technique has been utilized by Ebina et al. [20], with the intention of altering the HIV-1 RNA as well as controlling how it is expressed. After transfecting the CRISPR-Cas9 system targeting long terminal repeat (LTR) sequences into LTR-expressing dormant and induced T-cells, they found that the LTR gene expression was significantly absent, confirming that CRISPR-Cas9 was able to effectively cleave and mutate LTR target sites, inhibiting the expression of activated proviruses and reactivation of latent viral reservoirs. It was also found that this technique could eliminate internal viral genes from chromosomes in host cells. This technique has been employed by Hou et al. [21], in order to select the human CXCR4 gene, a secondary receptor of HIV, to enable CD4+T cells to develop specific resistance to HIV, inhibit HIV-1 infection, and delay the course of the disease by knocking down the expression of the somatic CXCR4 gene.

Positive-stranded, ssRNA viruses include the Hepatitis C Virus (HCV). Many powerful direct antiviral drugs (DAA) that target the proteins that make up the virus have been developed, and as a result, most infections with HCV—regardless of genotype—can now be cured with the right pharmaceutical treatments. Drug-resistant HCV genotypes can be targeted by disrupting the HCV genome with CRISPR-Cas9 technology. Price et al. [22] transfected cells with gRNA-FnCas9 targeting the HCV genome, which significantly reduced the expression of HCV proteins and ultimately inhibited HCV replication.

5. Conclusions

CRISPR-Cas technology is quickly becoming a valuable tool for detecting nucleic acids not only in animal viruses but also in plant viruses and bacterial typing. Both Cas12a and Cas13 have shown impressive accuracy, made virus detection and monitored possible through DETECTR, SHERLOCK, HOLMES, and other methods. These methods are gaining popularity due to their efficiency, affordability, ease of use, and quick results. They hold great potential for contributing to the cost-effective and efficient detection of viruses.

Furthermore, the recently uncovered class V Cas12a exhibits a reduced molecular weight compared to Cas9 and a higher count of target sites. These factors make the CRISPR-Cas12a technology more proficient in precise gene editing than the CRISPR-Cas9 technology. It is evident that the CRISPR-Cas12a technology has a bright future in advancing antiviral research, and it is expected to become a potent weapon in the fight against viral infections.

Although CRISPR-Cas has shown promise in genetically modifying organisms and antiviral therapies, its cutting method can pose risks and unintended effects on their original genomes. To ensure safety, it is recommended to use Cas9nickase due to its reduced off-target properties. Additionally, numerous viral genomic locations might need to be concurrently targeted simultaneously to prevent drug-resistant mutations and inhibit viral escape. When selecting gRNAs for the CRISPR-Cas technology, it's crucial to prioritize the most critical and conserved viral sequences. Similarly, when targeting host factors to combat viruses, it's important to focus on factors that are necessary for the virus but not critical for the host. Finally, monitoring the host's post-targeting activities is crucial to avoid any unintended consequences.

The potential of CRISPR-Cas technology for virus detection and antiviral research is promising. However, its rapid emergence has presented various challenges and limitations that require careful attention. Nevertheless, with continuous refinement and improvement, it is reasonable to believe that these obstacles can be mitigated or even eliminated, resulting in a safer and more effective CRISPR-Cas tool. The benefits of this progress will be significant, as it will greatly contribute to the progress and success of antiviral research, prevention, and treatment, ultimately promoting the health and well-being of society.

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Applications in gene editing in cell totipotency

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Abstract. Cell totipotency is that in multicellular organisms, each of cells contains all sets of genetic materials. Therefore, cells have the potential of developing into new individuals. According to tissue culture in plants, this has inspired biologists and they started conducting extensively in the field of cell totipotency for a long time. Moreover, scientists have made significant achievements in terms of the field in cell totipotency from embryonic cell division technology, nuclear transfer, induced pluripotent stem cells and so on. However, based on limitations in the development of technology, mechanisms of differentiation and dedifferentiation in animal cells are still unknown. The emergence of CRISPR technology in the twenty-first century has revolutionized an efficient method to research structures and functions in genes. Because of its durability and efficacy in genome editing, the CRISPR/Cas9 system has recently become one of the trendiest issues. What's more, this kind of technology also had a huge breakthrough in many fields, and it has typically been used to genetically modify pluripotent stem cells, which are then differentiated into specific cell types and used for functional study and even clinical transplantation. This review aims to introduce the general applications of CRISPR and expound its potential breakthroughs and mechanisms in animal cell totipotency, which may guide future research efforts in this area.

Keywords: CRISPR, differentiation, animal cell totipotency.

1. Introduction

In organisms that are multicellular, cell totipotency refers to the fact that each cell possesses all sets of genetic material. As a consequence, cells have the capacity to grow into new individuals. According to recent studies, which show that each plant cells have cell totipotency. That means they can develop into a new and intact plant from a single cell or partial tissue regenerations. Utilizing plant cell totipotency to develop plant cell tissue culture technology and virus-free plants. This not only has a positive effect on the benefiting economy. On top of that, it has wide-ranging implications for scientific research and plays a crucial role in preserving biodiversity by safeguarding endangered plant species. Biologists obtain inspirations from plant cell totipotency, which led to a chain of research in animal cell totipotency. This produced embryonic cell division technology, nuclear transfer technology and induced pluripotent stem cell technology. Besides, this also developed regenerative medicine, animal cloning, stem cell culture and various fields. All of these have huge important in research and medical significance [1].

Based on early studies, scientists have already revealed that the nucleus in animal cells maintains totipotency while they are growing. Furthermore, under the role of some factors in the oocyte cytoplasm, they can help animals to restore the ability which is cell totipotency in order to develop into a new

individual. In subsequent studies indicate that the result in gene selection is differentiation. During this process, some genes will be labeled due to the combination of methylation, acetylation or some miRNA. On the other hand, some substances located in the cytosol such as MPF can delete that mark. As a result, cells have the ability to restore totipotency. In conclusion, highly divided cells can possess totipotent iPS cells by adding some factors because they enable cells to differentiate for totipotency. Animal cell differentiation and dedifferentiation are extremely accurate and precise processes. Because of technological limitations, scientists have little understanding of the mechanisms of cell differentiation and dedifferentiation, nor do they fully comprehend the structures and regulatory processes of genes relevant to cell totipotency. Because of these constraints, the number of trials using reverse cell development is often modest. Consider Shinya Yamanaka's induced IPS cells. The experiment's success rate was only 1/5000. In recent years, conditions for experimenting with reverse cell development have become an increasingly popular topic. The primary focus of this guide is on totipotent genes and processes. However, the sheer volume of gene data implies that the research will most likely take a long time to complete. Therefore, it is important and expected to develop new technology in order to perform the experiment of reversing cell growth [2].

The CRISPR/Cas9 system is a powerful tool for genetic engineering that is composed of a small guide RNA (sgRNA) and a Cas9 protein that functions as an endonuclease. After the specific identification of the sgRNA, Cas9 protein will arrive at a certain location in the genome. Therefore, it can cut a double-stranded DNA (dsDNA), and later produce a double-strand break (DSB). However, there is a key point. DSB performs restoration by nonhomologous DNA end joining (NHEJ) or homology-directed repair (HDR). Moreover, NHEJ which is dominant in these processes has a high possibility of becoming mutant. Based on that situation, CRISPR/Cas9 acts as an efficient way to perform cutting in target genes. This method possesses a variety of advantages, like being easy to control and having a highly efficient characterization. Functions and impacts of targeted genes can be identified by cutting and breaking some of those genes. With the development of CRISPR technology, this led to a serious set of changes. For example, scientists not only can conduct gene excision of double-strand, but also evolved to single-base excision that does not involve breaks. This progress also means that both DNA and RNA can be altered to validate findings. It greatly furthers our understanding of mechanisms related to potentiation / differentiation within certain animal cells, and it holds great promise for future achievements in research on cell function and behavior [3,4].

This review combined contemporary research with findings from animal totipotency and CRISPR gene editing in order to explore the possible roles in genes and mechanisms of animal cell totipotency. What 'more, it offered guidance and instructions for cloning and tissue culture in the future.

2. Hundreds years of cloning-Cell totipotency

2.1. Performance on cell totipotency under natural conditions

The capability of a cell to fertilize other cells that have already been divided in organisms, such as sperm and spores, is known as cell totipotency. Because when animal cells mature into individuals, they possess all of their genetic material. In light of this, animal cells also possess cell totipotency. However, due to multiple constraints, it is challenging for them to demonstrate such a skill. It's important to note that animal cells that exhibit different totipotent behaviors [5].

Lower creatures like polyps and other coelenterates produce in a manner akin to fungi. The only difference is the size. Highly evolved animals, however, have a far reduced capacity to exercise that capacity when compared to lower organisms. The explanation for this is that the level of cell totipotency in animal cells varies. Strictly speaking, only the nucleus has cell totipotency in animal cells because the nucleus contains all genetic materials. The first experiment on the fertilized egg of an African claw frog was conducted in 1964 by the English scientist Gurdon, who demonstrated that the egg had cell totipotency. Even while the nucleus is capable of doing so, it has very little flexibility. Scientists' attention is once more attracted by the fact that lower animal cells have the capacity to build organs. Because of that result, scientists are excited about finding cell totipotency's ability in animal

cells, and start researching stem cells, artificial organs and clones. But there are still a lot of secrets that remain undiscovered. As a result, research on cell totipotency is still substantial and instructive.

2.2. Clone

Cloning exhibits cell totipotency at the highest level. Therefore, it's important to mention the word "clone" here. A cell can reproduce by an asexual process called cloning, which allows it to divide repeatedly. A highly divided cell's ability to clone reveals that the totipotency of an animal cell can be determined. This finding has not, however, received official validation.

From the beginning in the year 5000 BC, humans recognized that they could cultivate corn using other plants. On top of that, Gregor Mendel experimented with plant hybridization in the 1860s. This verified a fundamental theorem of heredity. The first known clone was created in 1902 by German scientist Hans Driesch, who was able to induce a monozygotic twin with a natural clone. After that year, Herbert Weber of the US Department of Agriculture created the term "clone." Hans Spemann's experiment was the primary fountain of inspiration for subsequent cloning. American scientists Robert Briggs and Thomas King established themselves based on the results of these experiments. An embryo developed after inserting the frog's zygote into the ovum's nucleus. For the first time, a human embryo could be reproduced using a nuclear transfer technique. It can also be considered a turning point for clones. The experiment was successful 104 times, and 27 out of 35 of the embryos turned into tadpoles.

At this point, the principal of cloning technology using animal nuclear transfer has been established. Following this, more and more animals have been reproduced using this method, such as the macaque. But successful rates of cloning in mammals are still low [6, 7].

Nuclear transfer technology suggests that early embryos have the ability for cell totipotency, although that capacity is considerably lower than the case for the early ones. Additionally, zygotes have a chemical in their cytosol that can alleviate restrictions on cell totipotency. The reasons for the reduction in cell totipotency that occurs as cells develop and the remedies for the problem. Scientists have struggled with these kinds of problems for a long time. Consequently, this article will expand on these questions.

3. CRISPR technology

3.1. The discovery of CRISPR technology

The discovery of CRISPR technology, as with other important scientific discoveries, can be regarded as a by chance. Francisco Mojica discovered CRISPR while studying an archaea in Spanish. After that, he found an intriguing phenomenon: this archaea contained multiple components. There would be no repetition of these 30-base chunks. Furthermore, the spacing was roughly 36 bases within two repeated bases. That's why Mojica saw this as CRISPR, or Clustered Regularly Inter-Spaced Palindromic Repeats.

Subsequent research investigations have revealed that the CRISPR system was actually part of a far more complex immune system seen in prokaryotes. The purpose of this technique was to combat viral infections. Furthermore, there are two components from the natural CRISPR/cas9 system: the cas protein and CRISPR. The cas9 protein cuts off a segment of the virus's DNA when it infects bacteria, and it will subsequently insert this portion into its own CRISPR region. After this procedure, the RNA will be replicated from the DNA and joined with the cas9 protein. Consequently, ribonucleoprotein (RNP) complexes were developed. This complex has the capacity to prevent bacteria from getting infected. Because of this mechanism, the RNP is able to recognize the DNA in the virus when it re-infects bacteria. Moreover, cas9 works to effectively degrade viral DNA [8].

The roles that distinct CRISPR system components participated in were demonstrated by French scientists Jennifer Doudna and Emmanuelle Charpentier. And such components replicate the mechanism by which bacteria aid in cutting off targeted DNA: the CRISPR system. The discovery by Emmanuelle Charpentier and Jennifer Doudna that gene editing be accomplished by altering the CRISPR system in bacteria and cutting off specific points in DNA molecules is significant.

3.2. *Mechanisms and applications of CRISPR-cas9*

With the CRISPR-cas9 system, edition of certain DNA sequences by cutting particular regions of the DNA can be achieved. There are two parts that function as distinct characters in this system. One is being used to cut the cas9 protein, while the other is being utilized to guide the RNA. A complex, RNF was created when these two parts were merged. It can pinpoint the location and erase DNA damage. Cas9 must first be found and then it has to connect with PAM, which is a specific genomic location. Guide RNA will then partially unzip the double-strand DNA after this step is complete. The truth is that in order to combine DNA on a specific sequence, guide RNA needs to be altered. If CRISPR identifies a target sequence, cas9 would be rapidly cleaved. Consequently, unzipping of the double-stranded DNA would result in enabling the target gene to be cut [9].

The DNA damage repair mechanism begins to function operating once the gene is cut. The telomeres created by the two ends of DNA are joined together during this process. Because the targeted gene is difficult to fully repair after deletion, the CRISPR-cas9 system is thought of as a tool for gene deletion. Additionally, the targeted gene's original function will be lost. By rendering one or two of the cleaved domains of the cas9 protein inactive, researchers were able to create a novel type of enzyme. And activate enzymes by using CRISPR targeting. Thymidine can be formed via a cytidine-specific mutation if cas9 is allowed to connect with deaminase. Gene editing will result in the transformation of mutant or aberrant illnesses into normal genes. Stop codon introductions is another way to accomplish that. Scientists do a great deal of experiments to increase gene expression. In one experiment, the transcription fusion factor bound to cas9 after the cas9 protein was rendered inactive. Activators that attach to guide RNA can also boost the expression of a gene. To increase expression, the activators bring transcription factors together. On the other hand, the crab-like domain, which is connected to the fusion expression of cas9 on the complex, allows scientists to gather more components if they wish to suppress gene expression. Scientists are able to observe the distribution of a specific DNA molecule in cells by combining the cas9 complex with a fluorescent protein molecule. This aids in visualizing the genome's three-dimensional structure.

Many fields have seen breakthroughs as a result of CRISPR. The uses of CRISPR technology by present researchers, however, are merely the tip of the iceberg. Although CRISPR research is still in its early stages, we think the technology will also revolutionize other industries.

3.3. *Application of CRISPR technology in the study of animal cell totipotency*

Considering CRISPR technology has moved forward, multiple other fields have made extensive use of this method of gene editing. Scientists have discovered that totipotent stem cells from animals can be used to generate artificial organs, particularly in therapeutic fields. However, from a legal and ethical perspective. Diseases cannot be treated using artificial organs. Stem cell therapy can be used to treat several illnesses, including chronic conditions. For instance, the treatment of Autoimmune Addison's disease (AAD) utilizing Adrenal Cortical Stem Cells. They can encourage the creation of steroids, which is the cause. Meanwhile, the use of CRISPR technology for organ transplantation offers promise for the medical community [10]. In 2022, YangLuhan, a Chinese scientist, performed the first successful transplant of a CRISPR-modified pig's heart into a human body. It indicates that the person was able to survive for 109 days. According to the latest paper, which was published by Nature, a machine survived for more than two years after receiving a transplant from a humanized porcine donor. In comparison to last year's results, lifetime increases significantly. Data demonstrates that during this time, scientists used gene editing 69 times and added 7 different types of human genes to a machine. This is one of the factors that extends the life of a living organism [11]. Numerous animals have had their genes altered up to this point. Additionally, this technology allows for the creation of new individuals. Black Angus cattle are an excellent example. By using these cows as breeding stock, scientists were able to create hornless cows by gene editing. Therefore, cows no longer are subjected to the pain of having their horns clipped.

With the development of technology and the efforts of researchers, many kinds of diseases can be cured, just like I mentioned above. This gives a lot of hope for both patients and scientists to deal with

disorders. However, the topic of applying CRISPR gene editing to human embryos has been argued for a long time [12]. This is because once scientists are allowed to reproduce these embryos, it will cause an extremely negative impact on the world. There will probably occur a phenomenon where richer people spend their money in order to make their children better than others. This will make the world much less fair than it is now. The use of CRISPR to alter human embryos is prohibited by law and morality. Despite the fact that solely the UK is authorized to undertake gene editing on human embryos. The process is only permitted for a short period of time, though [13].

4. Conclusions

Animal cell totipotency has been studied for many years by researchers. Scientists' efforts result in the change of macros into molecules such as DNA and RNA. However, due to technological constraints, a complete mechanism and theorem system for gene study remains unattainable. This turned into a bottleneck for scientists and researchers. The discovery of CRISPR-cas9 offers hope for the study of gene expression and functions. Furthermore, by modifying genes, scientists can carry out research on the locations and functions of genes through the genome. In the meanwhile, this essay analyzes the basic workings of CRISPR and its uses in several domains, including CRISPR application settings for potentially successful cell totipotency research. We are hoping that these ideas may help with future CRISPR research.

CRISPR has a significant impact on researching cell totipotency. The expressions of cell totipotency can be increased or decreased by altering expressions of activators and inhibitors in cells. This can be beneficial in a variety of academic domains. For instance, growing mature body cells can increase the totipotency of particular genes. since they are cultivable and can become necessary organs. Moreover, cell totipotency aids in the identification of aging-related modifications to the genetic makeup of genes. Scientists can therefore stop this from happening by blocking alterations in molecules that occur with age.

Author contributions

All the authors contributed equally and their names were listed in alphabetical order.

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Wearable biosensors and their applications in healthcare

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Abstract. The technologies used in medical care region could become more widely-discussed in the society. In the past, most biosensors were used in the manufacturing and engineering to increase the efficiency of production enable to meet the requirements of mass production, as the inventors expand the use of piezoelectric biosensors, their application in healthcare and virtual reality become important. Therefore, this paper collected and summarized the results of several previous research papers, explained the different working theories of many types of biosensors, and detailed discussed the specific biosensors used in these areas to look into this topic. The finding is that effectively use the piezoelectric materials (PZT) sensors could largely improve the medical level and the function of virtual reality. The wearable haptic feedback system can be used not only in these areas, but lots have not been explored. The medical level and other fields can all be improved even more in the future through the use of biosensors.

Keywords: biosensors, piezoelectric materials, healthcare

1. Introduction

Nowadays, as more and more intelligentially, there is a huge improvement in the application of different biosensors in medical care, industrial engineering, virtual reality and also technological products like smart watches, which gives a very efficient and convenient way in production and consumption. Today, many biosensors are used in hospital and research department, which largely increased the accuracy in diagnosing illness especially for common diseases with high mortality rate also decreased the total time duration. One of the most widely-used biosensors in medical care is the piezoelectric biosensors made from piezoelectric materials. In the 1960s, there is an initial assumption of wearable feedback system, it became in the spotlight as the inventor's research lasted for fifty years. Nowadays, inventors are focusing on choosing different materials of wearable haptic biosensors, aiming to produce a biosensor that has high sensitivity, is not easy to deform, which highly increased its practicability in different industries and fields to increase efficiency. This paper first explained the working principles of diverse biosensors, based on some previous papers with specific type of sensors, then summarized those existing research results and talked about the application of haptic biosensors specifically in medical care field, mainly the use of PZT sensors and aimed to provide reliable information for further research on wearable haptic biosensors.

2. The principle of biosensors

A biosensor is an analytical device that converts a biological response in an electrical signal by means of a transducer. And send the output to meet information transmission requirements, process, storage,

display, record and control. The object that has been measured can be detected by sensing element, then send it to transducer, finally the electrical signal can be produced. A sensor usually composed by two parts: sensing element and transducer [1]. There are several kinds of sensors; they all play essential roles in different area.

Electrical resistance strain sensor is a sensor that converts the physical quantity pressure to electrical signal output. The electrical resistance strain sensor with the resistance strain gauge as transducer is mainly composed by elastic element, resistance strain gauge which is pasted on it, circuit of output electrical signal and a compensation circuit. The elastic element is the key of the whole structure; there are several forms of it to raise the stability to detect the measured quantity. The principle of electrical resistance strain sensor is when the measured object can form an elastic deformation on the elastic element, the resistance strain gauge can detect the change of conversion from elastic deformation to resistance value. Next, the electrical signal can be output through circuits [2].

Heart rate sensor detects the changes in the arterial pressure or uses the property of blood which is it absorption of near infrared light, converts it to electrical signals. The most common method is photoelectric measurement. The photoelectric heart rate sensor sends out a beam of light. When the heart pumps, the light of specific wavelength will be largely absorbed by blood. The fluctuation of heartbeat can be measured by detecting the reflection light. Also, method of electrocardiosignal detection can be used in the sensors. Another way is to use high-specification sensors to capture the vibration of body cause by heartbeat [3].

Motion sensor is a sensor that fixed in specific area of a moving object; it can provide information of the location of the object, and monitor the physiological and motion information of a human body [4].

Photoelectric sensor is a sensor converts the changes of light intensity to electrical signals to control. It is composed by a transmitter, receptor and detection circuit [5].

Optical biosensors emit an optical signal. There are several types of detection. The first way is through a beam. Both transmitter and receiver are placed pointing face-to face, enable to create a straight light beam path. The second one is diffuse reflective. The light is reflected by the object when it is transmitted by transmitter and the reflection light is measured by the receiver. The third way is retro-reflective. The reflector reflects and the receiver receives the light beams transmitted by the transmitter. The light beam breaks when any object comes in between. Based on difference between light beam intensity and other parameters object can be detected or sensed at the receiver.

The most important sensor in wearable haptic sensory system is the piezoelectric sensor. It is the application of piezoelectric effect, which is the generation of electrical potential of voltage when mechanical strain is applied, or vice versa. A piezoelectric sensor is made from PZT, which is a crystal or a substance of a crystalline properties that can produce the voltage. PZT is a non-centrosymmetric crystal structure with a net non-zero charge in each crystal unit cell before applying mechanical stress. When pressure is applied to PZT, an electrical voltage is produced due to charge deformation, the titanium ion's position is shifted inside the unit cell and the development of electrical polarity takes place. As a result, the unit cell turns into an electric dipole. Some PZT materials are: Quartz crystal, topaz, Barium titanate, Polyvinylidene fluoride (PVDF)... For example, a piezoelectric microbalance biosensor has the quartz single crystal as its sensing unit, it can detect the change of mass because of the synthesis of antigen of a specific-protein complexes. It has application in detecting bacteria, virus, DNA and biochemical indexes. The working principle of virus detection using a piezoelectric biosensor is when a mass increases due to the interactions by the AC voltage decreases, probe antibodies are fixed on the piezoelectric material's upper electrode surface, the target antigen then binds with the probe antibodies changing mass on the electrode surface, change in mass leads to a time-dependent frequency shift that is represented by the frequency change of the material in the oscillation circuit.

3. The research of biosensors

According to the research on the BMD101 wearable heart rate sensor, it gives a lot of data to improve the application of BMD101 sensor, from data channel isolation, pure power supply, selection of ECG electrodes and software design [6]. These experiments show that the effect of directly using the DC power source in the notebook will be better than ordinary compatibles. An anti-interference measures for data transmission to a PC through a USB port is used, which is the ADUM4160 chip, as showed in Fig.1. Flexible fabric ECG electrodes are recommended as its excellent flexibility and electrical conductivity, especially for its good performance when it has a contact with a sweaty skin of human body. Finally, the system uses a wireless transmission module for data transmission, this greatly solved cross-talk of the PC to the BMD101 ECG sensor.

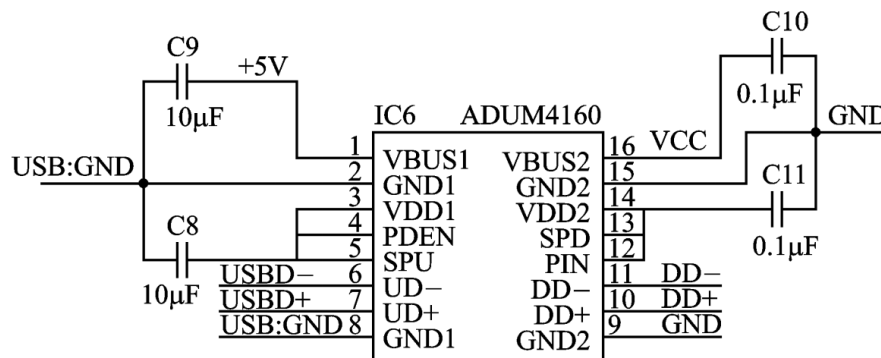


Figure 1. Digital signal isolation [6].

Luo et al. set a series of experiment to design a node for motion sensor [7]. Three quantities have been tested: heart rate, temperature and number of steps with distance travelled. The standard electronic sphygmomanometer, thermometer and test instruments is to measure the users' heart rate, temperature of the same test point and users with different speed and step size along a 5-metre-long straight line. Enable to compare the results of the practical circuits and measured results. As a result, very small differences have been showed in these experiments. The ADS1292 module was used to measure heart rate, the LMT70 module was used to measure temperature, and the MPU6050 module was used to measure the number of steps and distance travelled. The Bluetooth serial port module HC-05 was used to monitor the mobile data online. The system structure is simple, real-time display with great accuracy and stability.

An arched flexible piezoelectric sensor is used in the experiments based on [8]. In this literature, a motion evaluation system has been investigated. This system uses a PVDF flexible piezoelectric sensor to detect the human body impulse when exercising with different intensity and time. Detecting and measuring human body impulse signals through step test and spanning bike exercises. These experiments all show the system's feasibility, composed of ESP32 single chip microcomputer, PyQt framework upper computer.

Bai mainly explains the design and application of flexible piezoelectric sensors [9]. Adding PVA to the piezoelectric composite of PZT/MFC lowers the hardness and increases cellulose-based composites' flexibility. The results indicate that this sensor has higher output power and greater sensitivity and stability than normal piezoelectric sensors. In addition, this flexible piezoelectric sensor can be applied to wearable haptic systems. Different composition can be used to detect various things. For example, some can monitor plantar pressure and others can monitor muscle group pressure.

4. The application of haptic biosensors in healthcare and virtual reality

Electrical resistance strain sensors can be used in diverse sensory systems, such as load cells, torque, pressure, displacement, and acceleration [2]. Heart rate sensor is one of the most common sensors in

our daily life. Almost every smart watch contains wearable biosensors. Some brands occupy most of the market resources through their low prices of the smart watches [3]. Also, those motion sensors are assembled in wearable equipment such as smartwatches. It can record the daily exercises, sleep, diet and monitor the body health through these data [7]. Piezoelectric flexible pressure sensor can be used to detect pressure ulcers in diabetic complications prevent varicosity of the lower limbs and such medical and recovery fields [8]. There is a new biosensor has been investigated, which can detect the cerebral cancer via very small amount of blood. This type of biosensor uses surface-enhanced Raman spectroscopy to detect tumor derivatives. This device has a very high specification and accuracy, largely decreasing the time and workload when diagnosing the patients who suffered from cancers. The PZT materials are used for energy harvesting and biomedical electronics. The piezoelectric resonance has been applied to a point sensor. The sensor element consists of a cylindrical piezoelectric transducer, made of the ceramic material lead ziconate titanate and divided into two elements: one for vibration and the other for detection. Nanoribbons of PZT have been developed enabling in vivo measurements of soft tissue viscoelasticity. The device is made by a stacking and microcontact transferring method. An array of actuators is laminated on the skin and stimulated to generate tissue deformations that are measured by another set of the PZT sensors, to provide information on the mechanical properties of skin. The polar polymers, such as PVDF, can also be used in flexible ferroelectric skin to detect the temperature distribution on the human palm. By placing gold electrode arrays on the top and bottom of the film, the temperature sensor can map the temperature variation of a human hand. In addition, a PZT sensing element can be attached to a catheter due to its flexibility. The external electrode is made by the deposition of a thin film of copper. Catheter-based sensors have been also used to measure the real-time flow in biosensing applications.

There is a detailed introduction of the application of piezoelectric sensors in virtual reality glove system [10]. The glove can act as a text input device. There are many piezoelectric sensors embedded in the gloves, enable to detect the movements of players and send information of motion to VR via Bluetooth. The voltage produced by the piezoelectric sensor deformation gives out finger motion information (Fig. 2).

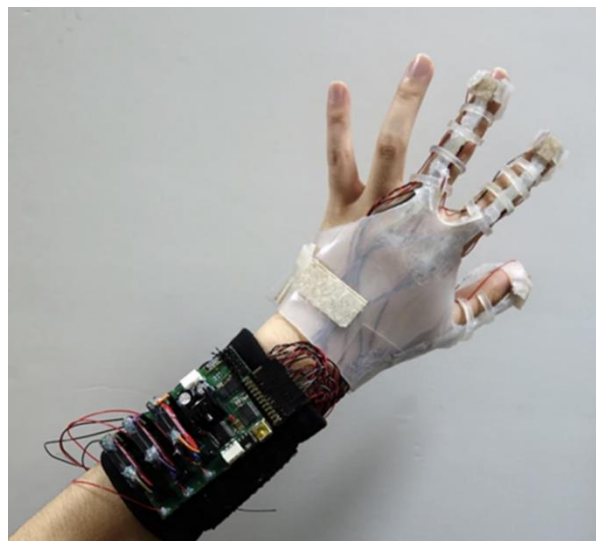


Figure 2. The appearance of the glove [10].

5. Conclusion

This paper gives the application of diverse biosensors in real life from different aspects such as healthcare, engineering, production, and so on, according to their basic rules of operating. This paper mainly introduced the working principles of electrical resistance sensors, heart rate sensors, motion sensors, photoelectric sensors, optical biosensors and piezoelectric sensors. Then concluded a few

developments of wearable heart rate sensors, motion sensors and also piezoelectric sensors, based on design and real experiments of the materials used, node and transmission module..., enable to get progress in the function of these sensors. Lastly, pointed out that the haptic biosensors play the important roles not only to the common industries that used to know, but also the developed technologies, like the actual feelings when players are using the virtual reality, through the researches of several biosensors, to rise the users' feeling experiences and happiness. This paper contributed to the applications of biosensors mainly in medical care, and is good for future research and development in this field. The future research could set up more experiments to enhance the theoretical results, enable to further rise the accuracy and reliability of the information, also let the biosensors exert the biggest influences to the whole world, especially to the convenience and efficiency in healthcare department, which could give the positive effects to the people's life expectancy, happiness index, working efficiency and even the economic growth due to the good health condition of the general public.

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Development and application of implantable electrical neural electrodes

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Abstract. Implantable nerve electrodes are a crucial neuro-engineering device that can play a pivotal role in the treatment of various neurological disorders. As the fundamental unit of the nervous system, neurons possess the capability to generate electrical signals, which serve as the primary means of information transmission. Implantable neural electrodes have the capacity to intercept and record these electrical signals, thereby providing invaluable insights into understanding nervous system functionality. Furthermore, these electrodes enable precise modulation of neural activity through targeted delivery of pulses to neurons. Surgical implantation of these electrodes within the nervous system allows for simulation or inhibition of neural signals with therapeutic objectives in mind. This article comprehensively reviews the significance and application of implantable neural electrodes in neuro-engineering while highlighting their immense potential in enhancing patients' quality of life across diverse neurological conditions. With further research and technological improvements, we can expect implantable neural electrodes to play an even greater role in improving the quality of life of patients, treating neurological diseases, and advancing neuroscience.

Keywords: Implants, Neural Electrodes, neurological disorders

1. Introduction

Neurological disorders have a profound impact on an individual's quality of life and overall health. However, the emergence of implantable neural electrodes as neuro-engineering devices has revolutionized the treatment of these diseases. By surgically implanting electrodes in the nervous system, neural signals can be simulated, suppressed, or modulated, allowing precise control and intervention of neural activity. This innovative treatment offers new hope and unprecedented opportunities for patients with neurological diseases.

The development of implantable neural electrodes has a long history, which can be traced back to the 1960s. At the time, scientists began to realize that electrical stimulation of the nervous system could alter the body's physiological and behavioral responses. This finding has stimulated interest in implantable neural electrodes to explore their potential in nervous system regulation and therapy. The earliest studies on implantable neural electrodes mainly focused on animal experiments. Scientists implant electrodes into animals' brains or other areas of the nervous system to electrically stimulate them and study their effects on behavior and physiological functions. These studies have laid the foundation for subsequent clinical applications and provided scientists with valuable data and insights. With the progress of science and technology, the research of implantable neural electrodes has

gradually expanded to the field of human clinical application. Currently, the market primarily provides mature implantable nerve stimulators such as deep brain stimulation (DBS), vagus nerve stimulation (VNS), and spinal cord stimulation (SCS). These devices have undergone significant advancements and are widely utilized in clinical practice. This article aims to comprehensively review the significance and application of implantable nerve electrodes in the field of neural engineering, discuss various types of nerve electrode treatment options, and explore their potential in treating nervous system diseases. A deeper understanding of these techniques by delving into the complexity of implantable neural electrodes and their effects on neural activity could pave new paths for discovering novel treatments for neurological diseases while contributing to the development of more effective therapies. This paper will contribute to advancing neuro-engineering research and improving patient outcomes affected by neurological diseases [1].

Implantable neural electrodes are neuro-engineering devices that are crucial in treating various neurological diseases. Electrodes that are surgically implanted into the nervous system can mimic or inhibit neural signals for therapeutic purposes. This innovative approach holds great promise in the field of neurology, showing great potential in improving the lives of patients with neurological disorders.

As the basic unit of the nervous system, neurons have the ability to generate electrical signals, which are the main way of information transmission in neural networks. Implantable neural electrodes are able to intercept and record these electrical signals, providing valuable information to understand the function of the nervous system. In addition, these electrodes can deliver electrical pulses to neurons, achieving precise control of neural activity.

Treatment options related to the level of implantable nerve electrical stimulation generally fall into 3 categories: stimulation, inhibition, and modulation.

Stimulation involves the application of electric impulses to stimulate or activate nerve activity. The method has been widely investigated and has demonstrated great success in many neurodegenerative disorders, including Parkinson. Perestelo et al. found that deep brain stimulation (a form of neural electrode-based stimulation) significantly improved motor symptoms and improved quality of life in Parkinson's patients [2].

Inhibition is to suppress or suppress abnormal nerve activity by providing accurate electric pulses with specified parameters. The technique has been demonstrated to ease the symptoms of illnesses like epilepsy. In a Closed Circuit Deep Brain Stimulation in Intractable Epilepsy Research, Nathaniel et al. demonstrated that a closed circuit DBS with an implanted nerve electrode can enhance seizure control and decrease adverse reactions [3].

In addition, regulation refers to the ability to modulate neural activity to restore balance and optimize function. Micera et al. proposed that electrical stimulation (ES) can partially restore motor and sensory functions. ES can be delivered through different interfaces and the invasive/selective ratio can be modified by changing the structure of the electrode [4].

In summary, implantable neural electrodes have revolutionized the field of neural engineering through their ability to mimic or inhibit neural signals. By harnessing the electrical activity of neurons, these electrodes provide innovative treatment options for a variety of neurological diseases. The treatment options for implantable nerve electrical levels typically fall into three categories

DBS: Electrical stimulation regulates neural activity by surgically implanting electrodes into specific brain regions, thereby alleviating symptoms. This technique is widely employed to treat Parkinson's disease, tremors, anxiety disorders, depression, and various conditions. Tourette syndrome (TS) is a complex neurological disorder characterized by motor and vocal tics. Although symptoms typically attenuate during adolescence and adulthood, some persistently experience severe manifestations. While the effectiveness of traditional treatments is limited, DBS has shown promising results in the management of the disease [5].

SCS: By placing electrodes surgically inside the spine, the technique is effective in blocking the delivery of pain signals and relieving chronic pain. It serves as an effective method for managing lasting pain. EMG is an electrical stimulus that is injected into the spine, which is used to stimulate the

spinal cord with electrical impulses. Millions of people around the world have seizures that are linked to irreversible brain or spine injuries that can result in long-term disability and lower quality of life. There are a number of therapies for the management of convulsions, but they all have their disadvantages. SCS can be regarded as an ongoing trial [6].

VNS: Electrodes are implanted in the neck region to modulate electrical activity within the brain by stimulating the vagus nerve. This therapeutic intervention is specifically indicated for certain types of epilepsy and depression management. VNS is an effective neuromodulation therapy for epilepsy. VNS is performed by implanting an electrode lead into the vagus nerve in the patient's neck to modulate brain electrical activity and reduce seizures' frequency and severity. The implantation of VNS is relatively simple and reversible, which can be adjusted according to the needs of the patient. Once implanted, the electrode leads undergo regular electrical stimulation through an external device and battery. The electric stimulus travels through the vagal nerve to the brain, which stops the abnormal electric activity, thus decreasing the probability of a seizure. A number of studies have shown that VNS is effective in non-responders to anti-epileptics [7].

2. Generation Epilepsy Management System

Kremen Vaclav et al. present an epilepsy management system (Figure 1) offers real-time, personalized treatment and monitoring for patients with epilepsy and physicians through the collaborative integration of brain implant devices, handheld devices, and cloud computing resources [8]. Their latest clinical studies have shown the effectiveness of seizure prediction, seizure detection, and therapeutic electric stimulation. The system builds a wireless connection between the handset and the cloud, enabling the user to connect seamlessly with the patient. Doctors have real-time access to the EEG information of the brain (waking/sleeping, pre-attack and epileptic fits), administration of electric stimulation and monitoring of the condition of the patient.

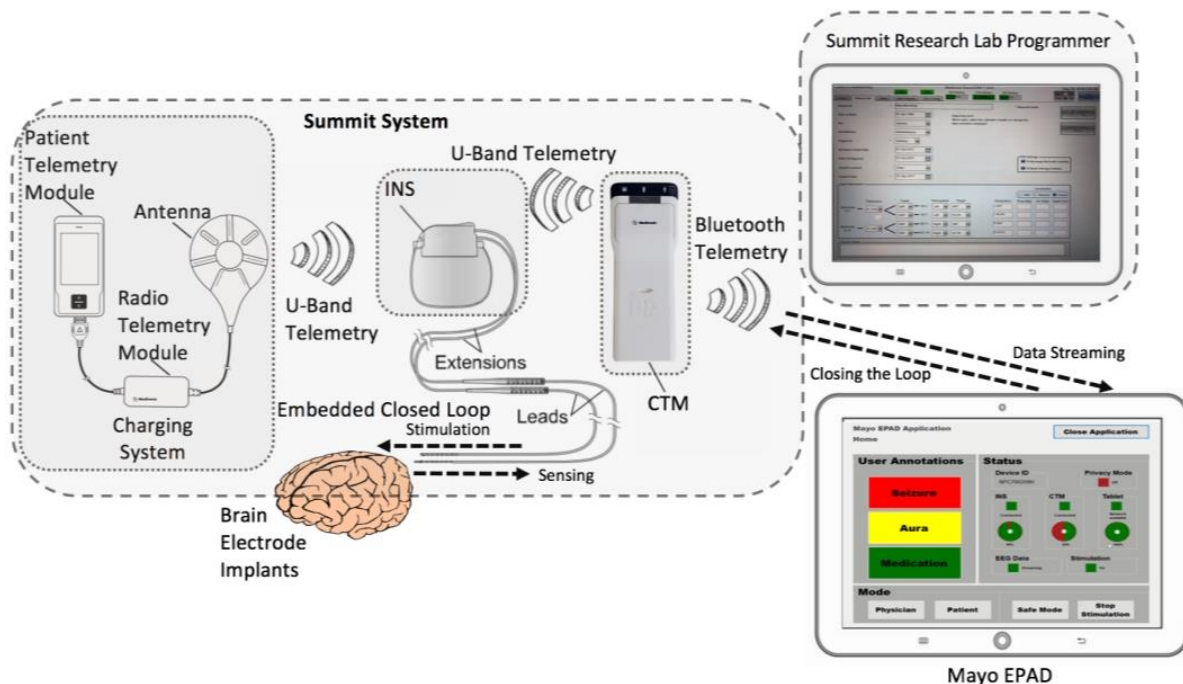


Figure 1. The Medtronic Investigational Summit RC + S System comprises an Implanted Nerve Stimulator (INS), Patient Telemetry Unit (PTM), Wireless Telemetry Module (RTM), Epileptic Assistance Apparatus (EPAD) and Research Laboratory Developer (RLP) [8].

3. Tuning Electrical Stimulation for Thalamic Visual Prosthesis

Amr Jawwad, et al. proposed a method for adjusting thalamic visual prostheses. This approach involves estimating an electrical stimulus that is equivalent to a given natural visual stimulus, resulting in a similar response when introduced into the lateral brain nucleus [9]. Furthermore, they employed artificial neural networks and autoencoders to control the firing of neurons in the LGN model (Figure 2) during the adjustment of electrical stimuli. The method consists of two stages: Firstly, when a visual stimulus is provided as input, the system predicts the neuronal response through the visual coding stage; the electrical stimulus decoder then utilizes these responses to estimate the required electrical stimulation for triggering such responses. This study introduces a novel approach utilizing machine learning techniques to optimize electrical stimulation and enhance simulated natural vision within lateral brain nucleus vision prostheses. Consequently, it improves visual function in patients with retinal degenerative diseases and provides new directions for future development of visual prostheses.

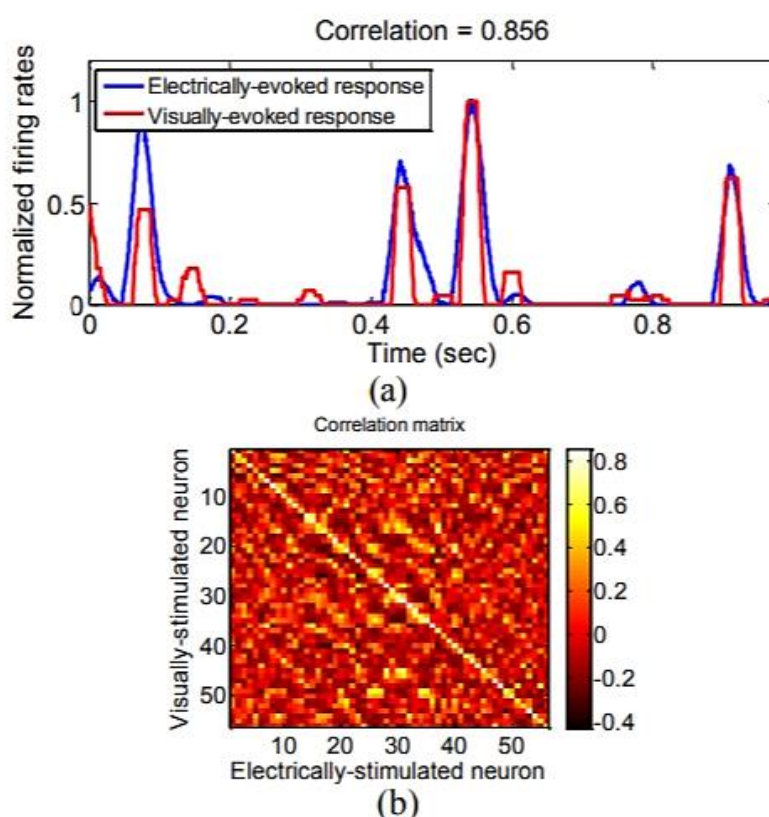


Figure 2. (a) The firing rates of a representative neuron in response to electrical stimulation are depicted in blue, while the firing rates elicited by visual stimulation are represented in red. (b) A correlation matrix illustrates the associations between the responses evoked [9].

4. Implantable Graphene-based Neural Electrode Interfaces

In their research, Ta Chuang Liu et al have presented an implanted multichannel nerve probe that can detect nerve chemistry and nerve electric signals in a multichannel way via a nonenzyme neurochemistry interface. A single step cycle voltammogram (CV) electrochemistry was used to synthesize the improved electrode on nerve tip. Gold was oxidized and GO was reduced at the same time, so that they could be tightly bonded by the electrostatic action of chloride (Cl^-). The RGO/ Au_2O_3 modified electrode exhibited significant improvement in electrocatalysis at a low deposition rate of 10 mVs⁻¹. Optical images are shown in Fig3. Nerve probe with or without rGO/ Au_2O_3 electrode. The results showed that the H_2O_2 reaction was fast under 5 s, and the detecting limit was below 0.63 μM . The H_2O_2 concentration of rGO/ Au_2O_3 electrode was $100.48 \mu\text{M} + 4.5 \mu\text{M}$ for 1 h in

an acute cerebral infarction model, which is significantly greater than that of the non-coated electrode. At the same time, SSEPs (SEP) were used to confirm the function of nerve activity. The new type of implanted probe with localized rGO/Au₂O₃ could serve as a rapid and reliable sensor for H₂O₂ or other neurochemistry [10, 11].

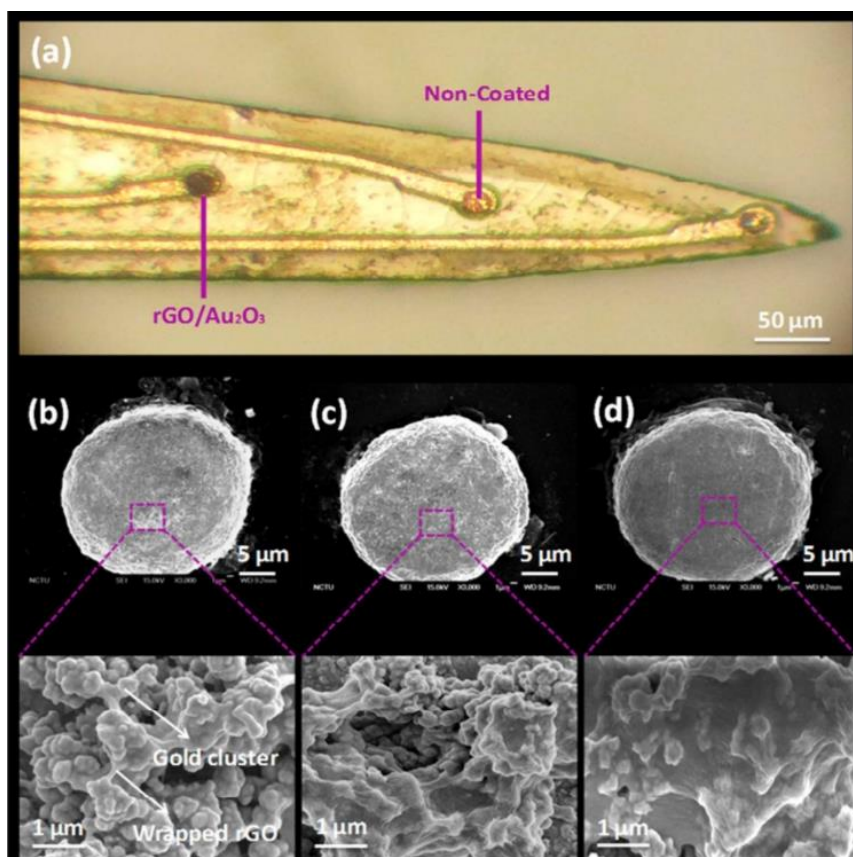


Figure 3. (a) Optical image of the neural probe coated with and without rGO/ Au₂O₃ electrodes. SEM images of the rGO/Au₂O₃ electrodes. (b) 10 mVs⁻¹, (c) 50 mVs⁻¹, and (d) 250 mVs⁻¹ [10].

5. Conclusion

In conclusion, implantable neural electrodes have revolutionized the field of neural engineering and hold tremendous promise in the treatment of various neurological diseases. These electrodes not only have transformed therapy but also significantly contributed to a deeper understanding of intricate workings of the nervous system. By intercepting and recording electrical signals, these electrodes provide invaluable insights into neural function and aid researchers in ending the complexity of the nervous system.

The field of implantable neurostimulators has made significant advancements in recent years, enabling real-time monitoring and personalized treatment for patients with epilepsy through the integration of brain-implanted devices, handheld devices, and cloud computing resources. Real-time data analysis allows for the adjustment of treatment parameters, resulting in improved seizure control and enhanced management of epilepsy. In the field of prostheses, researchers have achieved notable progress in refining electrical stimulation techniques for thalamic visual prostheses. This optimization of the electrical stimulation mode delivered to the thalamus has significantly improved patients' visual perception. Another major breakthrough is the development of an implantable graphene neural electrode interface which offers high electrical conductivity, biocompatibility, and precise recording and monitoring capabilities when implanted into the brain. This innovation provides new possibilities for understanding brain function and diagnosing nervous system diseases. Furthermore, integrating

implantable neural electrodes with wireless communication technology and miniaturized electronics enables closed-loop systems that dynamically adjust stimulation parameters to optimize therapeutic effects. This closed-loop approach enhances both efficacy and efficiency in nerve stimulation therapy summary, recent advancements in the field of implantable neurostimulators have expanded possibilities for more precise, efficient, and personalized neurostimulation therapies. Implantable neural electrodes have had a on the field of neural engineering while offering a novel approach to treating disorders within the nervous system. With continued progress in research and development, they possess significant potential to further advance our comprehension surrounding its intricacies while providing patients with more effective and personalized treatments in future endeavors.

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Optical biosensors based on microfluidic chip

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Abstract. Microfluidic chips have the advantages of small sample size, low detection cost, and short detection time. Combined with optical sensors, electrochemical sensors, and microwave sensors, they can be used to construct sensitive, repeatable, and portable detection systems. Optical biosensors are powerful analytical instruments that can measure biomolecular interactions in real-time and unlabeled manner. The combination of affinity and kinetics can be measured through high sensitivity. many optical biosensors have microfluidic control, making them extremely sensitive. Combining the concept of microfluidic technology, this article briefly elucidates the various cutting-edge research fields of microfluidic technology combined with optical biosensors, such as optical detection methods suitable for microfluidic chips, microfluidic biological immunofluorescence detection, the application of microfluidic technology in optical detection methods. It also prospects the future development direction and the challenges as well as strategies for addressing the problems in the development of the new generation of biological microfluidic sensors were proposed.

Keywords: optical, microfluidic chip, biosensors.

1. Introduction

With the rapid development of microelectromechanical technology, a wave of miniaturization manufacturing has emerged, and various new technologies characterized by miniaturization, high sensitivity, and intelligence have emerged. Microfluidic chip technology was born in this context. In recent years, microfluidic chip technology has gradually become a research hotspot in the current cutting-edge analysis field due to its miniaturization, low cost, and high sensitivity characteristics. Researchers integrate complex external fluid migration processes into micro chambers with scales ranging from a few micrometers to a few hundred micrometers, and use various microfluidic control technologies such as electrochemical reactions, electrostatic drive, and pneumatic control to achieve precise control of fluid inside pipelines. At present, this technology has been widely applied in various fields such as biochemical analysis, fluid control, and microfluidic optical chips. In recent years, optical analysis and detection systems have been widely used in material detection and analysis due to their integrated, high-precision, and high sensitivity characteristics. However, traditional optical analysis instruments have many defects such as large volume, expensive price, and poor adjustability, which cannot meet the current requirements of micro integration and become a barrier to the further progress of modern micro optical systems. Based on this background, researchers began to apply the concept of microfluidics to miniaturized optical switches, opening a new chapter in the study of miniaturization of optical systems. With the continuous deepening of research on microfluidic optical

systems, microfluidic chip technology and optical systems have further deepened their connection in various fields, such as detection and analysis, processing and manufacturing, and immunofluorescence analysis. In today's rapidly developing technology, it is believed that in the future, microfluidic technology will also open up a new research approach in optical systems. This article reviews the cutting-edge research fields of microfluidic technology in optical systems in recent years, mainly elaborating on optical detection methods that suit microfluidic chip and representative application fields combined with microfluidic technology such as protein immunoassay and heavy metal fluorescence detection.

2. Optical detection

2.1. Raman spectroscopy detection:

The combination of Surface enhanced Raman spectroscopy (SERS) and microfluidic chips for optofluid detection can achieve fast and non-destructive detection and analysis. Raman spectroscopy can characterize the vibration, rotation, structural characteristics and other spectral fingerprint information of material molecules. Compared to other spectral detection methods such as near-infrared spectroscopy, fluorescence spectroscopy, X-ray spectroscopy, etc., Raman spectroscopy has superior sensitivity and resolution.

Raman spectroscopy can semi-quantitatively represent the content and category of substances based on the Raman shift and intensity information expressed by different structural molecules. It has high detection sensitivity for both polar and non-polar molecules and is currently widely used in the analysis and detection of solid-liquid substances.

Surface enhanced Raman spectroscopy is an emerging advanced trace analysis technique with ultra-high sensitivity and unlabeled fingerprints. Stable, reproducible, high-strength, and highly sensitive SERS signals are the primary conditions for obtaining effective biosensing information.

Numerous studies have shown that controllable metal nanoparticle aggregates or nanoparticle arrays are crucial for generating the aforementioned SERS signals. In clinical immune testing, SERS biosensors can not only quickly detect a single biological protein marker, but also achieve simultaneous detection of sensitive and specific biomarkers based on solid-state SERS active substrates. Portable and intelligent microfluidic SERS biosensor detection equipment will be widely used in fields such as healthcare, environmental monitoring, and food safety.

For example, Castano et al. integrated microfluidic chips, optical tweezers, and Raman detection for real-time detection of the hemoglobin oxygen molecule conversion process of a single red blood cell [1]. Optical tweezers were used to bind a single red blood cell in a microfluidic pipeline, and Raman resonance spectroscopy was used for real-time detection, enabling synchronous changes between intracellular oxygen cooperation and spectral signals for scientific researchers. Huang et al. tested red blood cells and compared them with traditional detection methods, proving that the signal difference between normal and abnormal red blood cells detected through Raman spectroscopy is greater, and the detection sensitivity is higher [2].

Alexandra et al. used SERS to examine and evaluate the number and distribution of Silver Nanoparticles in living cervical cancer cells, combined with cell viability measurements [3]. The system schematic of this chapter is shown in Fig. 1 [3].

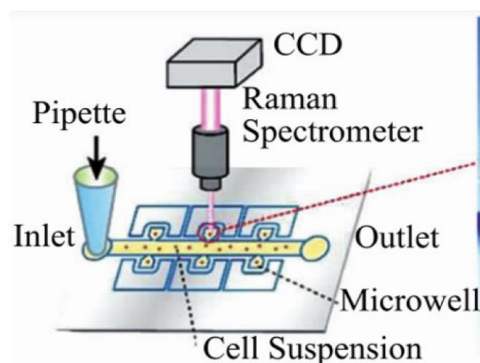


Figure 1. Schematic diagram of single cell SERS system based on microfluidic chips [3].

2.2. Refractive index detection

The refractive index of a material is a physical quantity that measures the speed at which electromagnetic waves propagate within the material. It is defined as the ratio of the propagation speed of electromagnetic waves in vacuum and a certain medium. The combination of microfluidic technology and refractive index detection methods is widely used in biomedical detection. The physical properties of samples are characterized through refractive index detection, which is suitable for certain analytes that cannot be detected by fluorescence spectroscopy, such as sucrose, polyethylene glycol (PEG), and other substances without fluorescence characteristics. The refractive index of cells, as a physical property of the cell itself, varies with the size of the cell volume and the diversity of surface proteins. In addition, the refractive index of cells is closely related to the microenvironment in which they are located, and real-time detection of the current physiological state of cells can be achieved based on changes in the refractive index properties of cells.

For example, Liu's team has established a small refractive index microfluidic detection system. In this study, optical fibers are used to position laser focused targets on the surface of individual cells, thereby achieving refractive index detection of individual cells. This chip system integrates three functions: Bragg grating, cell microenvironment regulation, and droplet separation. It not only achieves non-destructive online refractive index detection of living cells, but also avoids changes in refractive index detection values caused by external uncertain factors that alter cell physiological characteristics [4-6]. The system schematic of this chapter is shown in Fig. 2 [7].

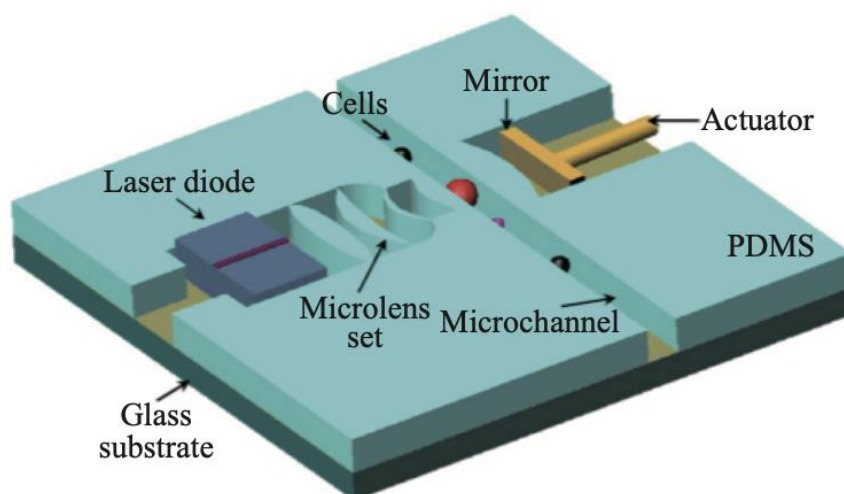


Figure 2. Schematic diagram of microfluidic refractive index detection chip structure [7].

3. Fluorescence analysis

3.1. Protein immunoassay

Protein immunoassay is based on specific reactions between antigens and antibodies. Conventional immunoassay includes three types: direct competition, indirect competition, and sandwich method. The testing principle is based on the specific adsorption between the antigen or antibody loaded on the solid-phase carrier and the reactant in the sample, thereby achieving the function of capturing the target substance. Then, the dynamic information of the target protein content to be tested can be obtained by comparing it with the linear correlation curve of the standard substance [8-10]. This principle is also widely applied in the field of microfluidic protein detection.

For example, Deng et al. used microfluidic chips to generate micro droplets carrying T cells, and then measured the protein content using immunocapture microspheres and fluorescent secondary antibodies [10]. In addition, immune capture microspheres and fluorescent labeled secondary antibodies can also be directly fixed in microfluidic pipelines using magnetic force or design microfluidic screening structures. By detecting the fluorescence on the surface of the captured microspheres, the content of the target protein in the sample can be obtained. At present, the microfluidic immunofluorescence detection platform designed based on microfluidic technology and combined with immune capture microspheres has been widely used.

3.2. Heavy metal fluorescence detection

Fluorescence detection is also commonly used to detect trace levels of heavy metal ions. For example, Bian et al. used microfluidic chips to measure the concentration of trivalent chromium through online fluorescence derivatization and laser induced fluorescence light, Spectral analysis of Cr (III) [11]. The combination of this self-assembled portable fiber optic fluorescence spectrometer and microfluidic chip enables the detection of Cr (III) concentration in actual water samples. Finally, the chemical sensor exhibits the advantages of high sensitivity, stability, fast response, and low sample consumption. Peng et al. established a simple, portable, and recyclable method for real-time detection of mercury and lead ions by using test paper based on gold (Au) nanoclusters as a fluorescence sensor [12]. This work starts with the structure of microfluidic chip fluorescence detection systems, and elaborates on the development and improvement of microfluidic chip fluorescence detection systems from the aspects of excitation light sources, light transmission assistance methods, and detectors. With the rapid development of life sciences, medicine, and pharmacy, microfluidic chip detection technology is increasingly being applied in various disciplines, and the requirements for its detection performance are also increasing, such as requiring low sample consumption, high analytical flux, fast analysis, high detection sensitivity, and good selectivity.

4. Conclusion

This article starts with the optical detection methods and applications of microfluidic chips, and elaborates on the application and development of microfluidic chip fluorescence detection systems from aspects such as Raman spectroscopy detection, cell refractive index detection and fluorescence analysis of proteins and heavy metals. With the rapid development of life sciences, medicine and pharmacy, microfluidic chip detection technology is increasingly applied in various disciplines, and the requirements for its detection performance are also increasing, such as low sample consumption, fast analysis, high analytical flux, high detection sensitivity, and good selectivity.

With the continuous development of analysis and detection technology, although microfluidic chip detection technology combined with optical biosensors has made certain progress, there is still some development space: for one thing, through integrated microfluidic systems combined with optical tweezers, nanotechnology and other means, the detection process can be more simple and fast. In the future, other detection technologies can be integrated to further improve detection resolution and signal range. And the reagents used for detection, such as enzymes, antigens, antibodies, etc., have high environmental requirements and are difficult to obtain and use immediately, lacking convenience.

For the other, microfluidic optical detection is also developing towards miniaturization. By miniaturizing the light source, detection module as well as analysis unit, the volume of the microfluidic chip detection system is further reduced, improving convenience.

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Neuromodulator approaches to depression: The potential of transcranial electric stimulation

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Abstract. Depression, a widespread mental health disorder, presents significant challenges in diagnosis and treatment. Amidst the myriad treatments, non-invasive Transcranial Electric Stimulation (tES), encompassing Transcranial Alternating Current Stimulation (tACS) and Transcranial Direct Current Stimulation (tDCS), has emerged as a promising intervention for Major Depressive Disorder (MDD). This review delineates the mechanisms of these modalities, their distinctions, and their potential to modulate neural oscillations and influence cognitive functions. Studies showcased the efficacy of these techniques in ameliorating depressive symptoms, with EEG and fNIRS employed to gauge their neurological impact. Observations suggest the potential in modulating for specific brain rhythms to enhance memory functions in older adults and influence working memory processes. The dynamic interplay between tDCS and tACS in altering cortical activities and their interaction with neurotransmitters like GABA further underscores their potential. However, the need for extensive research, particularly double-blind randomized control trials, remains paramount to ascertain their safety and efficacy. This review provides a comprehensive insight into the prospective role of tES in the clinical landscape of MDD treatment.

Keywords: Transcranial Electric Stimulation, Major Depressive Disorder, neural oscillations, memory functions, neurotransmitters.

1. Introduction

1-3% of people in the US suffer from MDD and are resistant to different treatment trials. Major depression causes the disability of millions of people as well as brings an enormous financial loss to society. One of the treatment solutions for treatment-resistant depression is neuromodulation, which offers hope to people for recovery [1]. Being depressed and having a risk of depression are caused by negative biases, lack of positive biases, self-referential processing, interpretation, attention, and memory, as well as the use of maladaptive cognitive emotion regulation. According to recent studies, limitation in cognitive regulations of mood-related content is the base of these cognitive functions. Abnormalities of memory, sensation, behavior, and personality are syndromes that often are results of brain disorders [2]. According to the World Health Organization, death from suicide each year reaches around 800,000 due to mental health issues [3]. There were developed criteria for diagnosing brain disorders and their pharmacological and psychological treatment. Unfortunately, understanding the neural foundation of the brain and the processes involved in these diseases is limited. The low efficiency of pharmacological treatment of certain brain disorders made scientists look for alternatives. The brain stimulation of

neuropsychiatric patients to modulate plasticity of their brain by different means of invasive and non-invasive methods [4-6]. Invasive neuromodulation using pharmacological interventions targeted microsimulation, optogenetics, etc., were employed to target brain function with spatial precision [5]. On the other hand, the way to modulate the brain without opening its skull is non-invasive brain neuromodulation (NIBS). Transcranial magnetic stimulation(TMS) and transcranial electric stimulation(tES) are the main methods of NIBS, which treat brain disorders [3].

What is: The foundation concepts of TMS and tES involve producing a potent current that activates neurons, affecting ion pathways and concentration differences to adjust the neuron's electrical balance. Three tES methods include transcranial direct stimulation(tDCS), transcranial alternating current(tACS), and transcranial random noise stimulation(tRNS) [8, 9]. The difference between them is the method of delivery of electric current.

Mechanisms: Transcranial alternating current stimulation(tACS) is a form of non-invasive deep brain stimulation with minimal side effects. Using an electric present influence on the brain's cortex enters brain oscillations and induces long-term synaptic plasticity to treat various psychiatric disorders. Cognitive neuroscientists have studied and examined tACS; however, its application of method was only recently used in psychiatry clinical trials [2]. tACS delivered low-amplitude electric current and was used for investigating the causal role of neural oscillations in cognition. Another neuromodulator approach that can influence neural systems function, cognition, and behavior is transcranial direct current stimulation(tDCS). Evidence shows that tDCS could impact various psychiatric symptoms, particularly depression and schizophrenia. Electrodes connected to the scalp delivered homogenous electric current in the range of 1-2mA, representing tDCS [10-12].

Interestingly, the current delivered as oscillations with specific frequency and stimulation amplitude is defined as tACS. tRNS is different from the other two methods by using random current amplitude. Modulation of ongoing neural oscillations and neuroplastic effect is a principle of tACS. On the other hand, membrane polarization-influenced neuronal excitability is the central concept for tDCS. There is a need to formulate definitive guidelines for tES, specifying the duration of modulation, intensity, and location of electrodes.

Transcranial direct current stimulation(tDCS) could also change neural system functions, cognition, and behavior. Evidence shows that tDCS could impact various psychiatric symptoms, particularly depression and schizophrenia. Nevertheless, research faces different challenges regarding how the method influences the effects, with no clear understanding of its effect on neural activity, functioning, and cognition of human beings. The essential points of the review are identifying future research priorities into MDD, such as mechanisms of action of tDCS and the potential of its clinical efficacy are the essential points the review. Constant monitoring of the progress of the crucial areas that containing data about advantages of using tDCS and its effectiveness during task training on prefrontal cortex could enhance behavioral performance. In the end, effective treatment of psychiatric disorders depends on an empirical study explaining the impact of tDCS on the CNS and its behavior [8].

It is essential to accurately assess neural activity in the brain, especially when using EEG for brain-computer interface objects. EEG is the oldest noninvasive neuroimaging modality, which is portable and has a higher temporal resolution as well as transcranial near-infrared stimulation(tNIRS) that measures oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) [9].

2. Perspective of Clinical Research

Outcomes (clinic/research): Symptoms of depression were improved using tES, an electrical stimulation therapy modality. EEG and fNIRS evaluated neurological effects and assessed therapeutic performance from hemodynamic changes. The power spectrum of the alpha band was used to evaluate the differences before and after stimulation. There were alpha bands with different ranges of 8 to 12 Hz, 8 and 13Hz, or 7.5 and 12Hz. Delta, theta, and gamma frequency bands of event-related potential (ERP) were also analyzed as components of the EEG-derived characteristics. Further data was gathered on the placement of anode stimulations for both tACS and tDCS, specifically in the left dorsolateral prefrontal cortex (DLPFC), and features were extracted from the alpha frequency range . These data suggested that the

left DLPFC is associated with right prefrontal hyperfunction and dysfunction of brain plasticity of depression.

A study using fNIRS revealed enhanced working memory performance and HbO in 26 patients with post-stroke depression after undergoing a 20-minute tDCS treatment with a 2-mA current on the left DLPFC, with the return electrode positioned on the right DLPFC. Another study was applied tACS at 10 and 40Hz in the DLPFC to examine the potential and effectiveness of altering alpha oscillations [10]. After 2 weeks of application, the 10-Hz tACS protocol was observed to decrease the intensity in the alpha frequency. Simultaneously, the current of 2 mA for 40 min was applied in one tDCS study, which was received thrice weekly for 6 weeks. There was an improvement in behavioral performance (emotional state and cognitive processing); however, brain activity assessments (frequency of distribution and ERP) had limited impact following the intervention. The exciting aspect of the studies was the different duration protocols and their effect on depression treatment. Short-term tDCS treatment protocols (5 sessions) positively affected the depression score and the power spectrum, but the improvement was sustained for a limited time of 4 weeks. Long-term tDCS treatment protocols (15 sessions) suggested improved mood and cognition in 50-60% of people with depression [11]. In addition, there were demonstrated that reduced ERP and frequency distribution (e.g., delta, theta, and alpha wave ranges) [12] by following tDCS protocols with lower intensity (1 and 5 mA) [13]. Specifically, combining positive psychotherapy with tDCS treatment protocols significantly influenced depression. Also, three months of follow-up exhibited a noticeable impact of combined therapy over tDCS alone [9] [12]. Non-invasive neuromodulation, tACS, developed guidelines can improve long-term and auditory-verbal working memory. Applying synchronous low-frequency neuromodulation to the parietal cortex showed enhanced working memory on days 3,4, and 1 month post-intervention. Similarly, synchronous high-frequency neuromodulation in the prefrontal cortex led to improved long-term memory on 2-4 and again after 1 month of the intervention. Based on memory-focused circuits' specific spatial and spectral characteristics, neuromodulation can effectively and continuously tackle the brain's adaptability.

Exploring distinctive rhythmic patterns in specific brain areas and exploiting these mechanisms by non-invasive methods such as tACS are essential tools to selective improve memory function in older adults. Working memory, long-term memory and free recall are considered to be influenced by oscillatory patterns in the theta and gamma frequency bands.

The scientists tested the hypothesis of improvement of auditory-verbal working memory function by modulation of theta rhythms in the inferior parietal lobule (IPL) and improvement of auditory-verbal long-term memory function by modulation of gamma rhythms in the dorsolateral prefrontal cortex (DLPFC). A set of experiments was conducted, which showed that memory could be improved through neuromodulation of functionally specific brain rhythms [14].

Paring tACS and electroencephalographic (EEG) to measure the functional connectivity of neural networks has great neuromodulation potential and lasting behavioral effects. Also, focusing on the brain areas with tACS at the theta wave range influences working memory processing and capacity [15].

3. Discussion

Resetting brain oscillations by the electrical current emphasizes its safety and efficiency and enhances behavior outcomes, according to findings of the clinical studies. Researchers are utilizing the brain circuit's responsiveness to tACS as a valuable instrument for the diagnosing, clarifying, and prognosis psychiatric disorders. Nevertheless, there are unresolved questions that future double-blind, randomized controlled trials could address by identifying response markers and adjusting for various complicating elements [16]. Customization of tES treatment protocols for patients with neurological and mental disorders offered the future direction by employing an integrated brain stimulation approach to maximize effectiveness and precision. Usually, there are extraordinary abilities of tDCS to induce excitability/inhibition and alter certain regions' cortical activity but limited to no abilities to modulate the specific oscillation frequencies. It has been reported that psychological mechanisms of tDCS have decreased gamma-aminobutyric acid(GABA) and elevated levels of glutamate/glutamine [17].

Decreased GABA levels have been associated with alterations in resting state connectivity. These findings have shown changes in functional connectivity after applying tDCS treatment.

On the other hand, tACS has the potential to generate a large number of neuronal firing events with externally introduced oscillations[18]. The most effective entrainment of tACS has been introduced when endogenous oscillation matches the stimulation frequency. The observed reliance of tACS on neural conditions might have been explained by this observation. In addition, tACS intervention suggested changes in GABA levels [19]. As a result, changes in functional synchronization of the brain would lead to alter following stimulation. The idea of using functional connectivity as a biomarker to monitor the tES effects might have been for future studies.

Most of the tES are not approved by the FDA for treating psychiatric disorders, which require authorization from licensed healthcare professionals. Also, instructions and guidelines on properly using these devices must be clarified and often confused. According to supervised and published research, there are no safety issues while using tES devices; however, they are not confirmed to have similar results if used individually and unsupervised. Unsupervised application of tES might cause potential injury, unforeseen cognitive impacts, and interference with other treatments. In addition, commercial and do-it-to-yourself (DIY) construction kits for tES must meet some electronic safety standards[20].

4. Conclusion

Depression, notably Major Depressive Disorder (MDD), continues to be a global health concern with enduring ramifications for affected individuals. The quest for effective interventions has directed attention to non-invasive neuromodulatory techniques, specifically Transcranial Electric Stimulation (tES). Within this spectrum, transcranial alternating current stimulation (tACS) and transcranial direct current stimulation (tDCS) stand out for their potential to offer symptom relief. Underscored by their capacity to modulate brain oscillations, these modalities are redefining therapeutic approaches. Preliminary research and clinical trials have showcased their ability to address cognitive dysfunctions, neural connectivity issues, and related symptoms of MDD. This review underscores their mechanistic insights, potential advantages, and clinical applications. However, like any burgeoning field, there is an urgent need for more exhaustive, rigorous research. It involves understanding long-term effects, establishing standard treatment protocols, and ensuring safety and efficacy for diverse patient profiles. As the medical community gravitates towards these innovative techniques, the overarching goal remains clear: to provide reliable, effective, and patient-centric solutions for MDD, thus enhancing the quality of life for millions grappling with this disorder.

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Immunotherapy and combined treatment in gastric cancer

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Abstract. Gastric Carcinoma (GC) ranks as the fifth inducing cause for cancer incidence, and it's the fourth inducing reason for cancer-related mortality. Traditional treatments, which involve a combination of chemotherapy and surgical resection, often yield unfavorable prognoses for gastric cancer patients, especially for those with advanced stages or metastases. Immunotherapy has recently emerged as the significant focus in the treatment of advanced gastric cancer, particularly for patients with MSI-H/dMMR. Medications targeting PD-1, such as nivolumab and Keytruda, have gained marketing approval in recent years. Additionally, some biomarkers like HER2, FGFR, and VEGF have shown positive efficacy in clinical research. However, resistance to immunotherapy remains a significant challenge, and the effectiveness of these pharmaceuticals often lasts only about a year for patients suffering advanced gastric cancer. Consequently, there's a need to explore new immune checkpoint inhibitors and discover novel combination therapy approaches. This review is aimed at introducing several latest immune checkpoint inhibitors currently in clinical phases or in pre-clinical stages, alongside innovative combination strategies for treating gastric cancer.

Keywords: Immunotherapy, gastric cancer, combined therapy

1. Introduction

The gastric cancer is a kind of malignancy tumor, and many patients are diagnosed with terminal cancer due to atypical clinical symptoms [1]. For patients who discover the gastric cancer early without tumor metastasis, the clinical surgical resection is a common therapy strategy. But unfortunately, many patients experience recurrence after the surgery. For advanced gastric cancer, only patients with HER-2 positive can be treated with trastuzumab accompanied with adjuvant chemotherapy for the first-line treatment [2]. Thus, new target spots is in need to be discovered and new medicine targeting new spots is in emergency to be used for first line treatment. Immunotherapy is a hot spot recently in cancer treating, some immune checkpoint inhibitors (ICI, targeting at PD-1 and PD-L1 spots) are gradually used for advanced gastric cancer treatment [3]. But the immune tolerance also arises and the median survival for advanced patients is merely extended for approximately two months compared with chemotherapy alone [4].

Thus, new treatment plans are in great demand for curing advanced GC, aiming in prolonging survival time of the patients and enhancing their quality of life. There are two ways: one is developing more immune checkpoints inhibitors (such as the ones targeting at CTLA -4 and LAG-3), the other is improving the inhibitory state of tumor microenvironment to confront the reality of immunotherapy tolerance (such as the Sting agonists). In this review, some new immune checkpoint inhibitors and some

Sting agonists will be introduced and some combined therapy (such as immunotherapy combined with targeting therapy and immunotherapy combined with chemotherapy) will be discussed aiming to establish a new treatment plan in advanced cancer.

2. Immune checkpoint in gastric cancer

2.1. PD-1/PD-L1

2.1.1. PD-1/PD-L1 structure. PD-1 (programmed death-1) is an important immunosuppressive molecule, which is a transmembrane protein, it contains an immunoglobulin variable region domain in extracellular parts, a transmembrane region, and within the cells, an immune receptor tyrosine-based inhibitor motif (known as ITIM) and an immune receptor tyrosine-based switch motif (known as ITSM). PD-1 belongs to the CD28/CTLA-4 family of co-receptors, with poor conservative gene sequence [5]. It plays a role in preventing excessive immune activation, however, the tumour cells can use its PD-L1 to unite PD-1 to avoid immune defences (Figure 1). PD-1 is mostly expressed in activated CD8⁺, CD4⁺ T cell, B cell and NK, DC, natural killer thymocyte cell [6].

PD-L1 (programmed Cell Death-Ligand 1) also named as CD274 and B7-H1 is a 40kDa transmembrane glycoprotein that is made up of extracellular segments, hydrophobic transmembrane segments, and short cytoplasmic regions. There is one immunoglobulin constant region (known as IgC) and one immunoglobulin variable region (known as IgV) in the extracellular domain. The cytoplasmic region in PD-L1 comprises a protein kinase C phosphorylation site [5]. PD-L1 is mainly expressed in T cells, B cells, dendritic cells (DC), macrophages, mast cells, fibroblasts, and mesenchymal stem cells, while on many kinds of tumor cells it is also highly expressed to bind with PD-1 and escape from the attack of active T cells [6].

2.1.2. PD-1/PD-L1 medicine. Many inhibiting medicines are designed to prevent the PD-1/PD-L1 pathway (Figure 2). Pembrolizumab is an antibody attacking PD-1, which got US FDA approval for use in many cancers and also got FDA's accelerated approval for curing the advanced gastric cancer [7,8]. And another approved inhibitor of PD-1 by FDA is Nivolumab. There are also other PD-1 medicine, such as dostarlimab, Xindiliximab injection, Treprizumab injection, Carolizumab injection, cemiplimab injection. While Atezolizumab, Avelumab, Durvalumab are some examples of inhibitors used for suppressing PD-L1. While these PD-1 and PD-L1 antibodies haven't got the approval from FDA in treating gastric carcinoma.

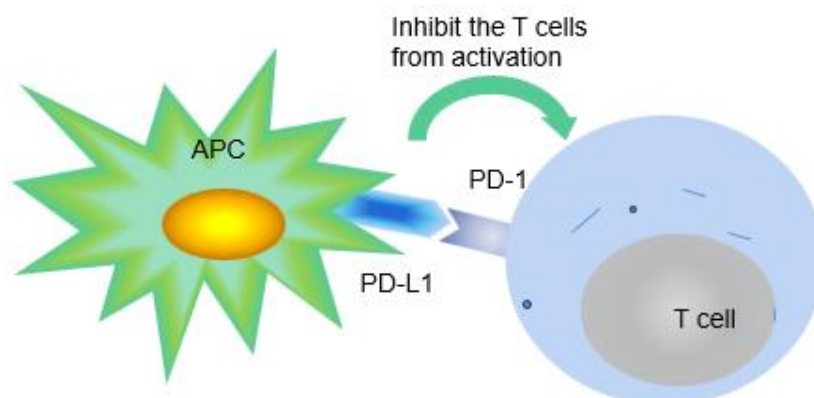


Figure 1. In naïve ways, PD-L1 on APK can bind with PD-1 on T cells thus to suppress the T cells from inordinate activation in case they attack the normal tissues.

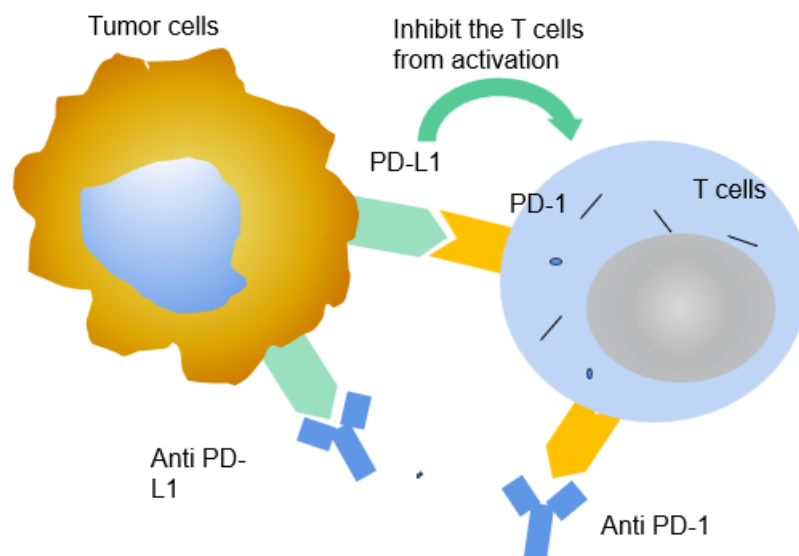


Figure 2. Some tumour cells also have PD-L1, which can lead to the misidentification for T cells. The T cells are deactivated owing to the bind between PD-1 and PD-L1, thus cease to assault the tumour cells.

2.2. CTLA-4

CTLA-4 (Cytotoxic T-lymphocyte antigen 4) is also expressed on the activated T cells, when CTLA-4 (CD152) binds to B7 (include B7-1 ligand and B7-2 ligand) on the surface of antigen presenting cells (APK) with a higher affinity than CD28 on T cells, it can competitively inhibit the binding of B7 to CD28 on the surface of T cells and prevent the T cell to be activated (Figure 3) [9]. There are some CTLA-4 inhibitors that are still on clinical tests for gastric cancer and especially the unite of CTLA-4 and PD-1 inhibitors is a focus these years (Figure 4). The AK104, which is an CTIA-4/PD-1 antibody, is in phase II to cure the patients with advanced gastric carcinoma locally by Peking University, which is highly expected [10]. Also, AI-061 which is also a co-formulation drug product for 1:1 ratio PD-1and CTLA-4 is recruiting volunteers for Phase one test for gastric cancer treatment [11]. Another clinical test is on Phase IA/IB/II to recruit people to test the ONC-392, which is a humanized anti-CTLA4 IgG1 monoclonal antibody for gastric cancer, also combined with Pembrolizumab to see it's effect [12].

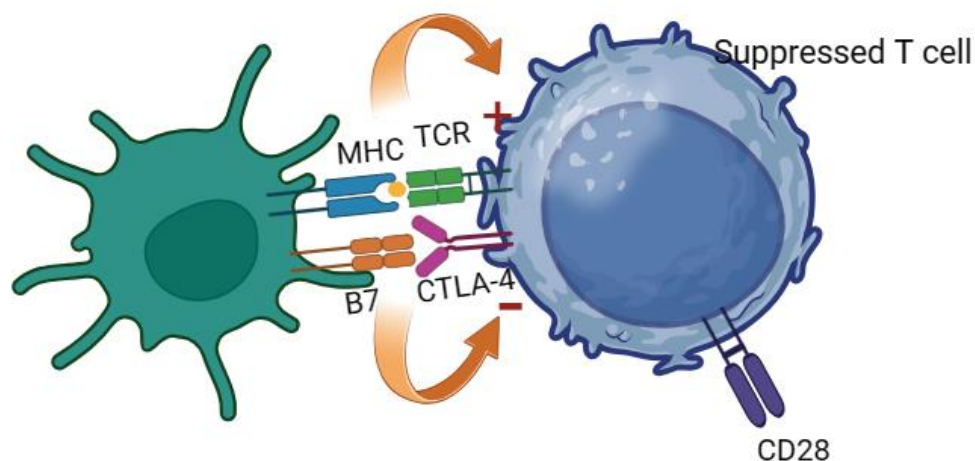


Figure 3. The TCR (T cell receptor) can bind with the MHC (major histocompatibility complex) and can activate the T cells, while with the aid of bind between B7 and CD28. The CTLA-4 has a higher affinity than CD28 with B7, thus the binding between CTLA-4 and B7 can suppress the T cells.

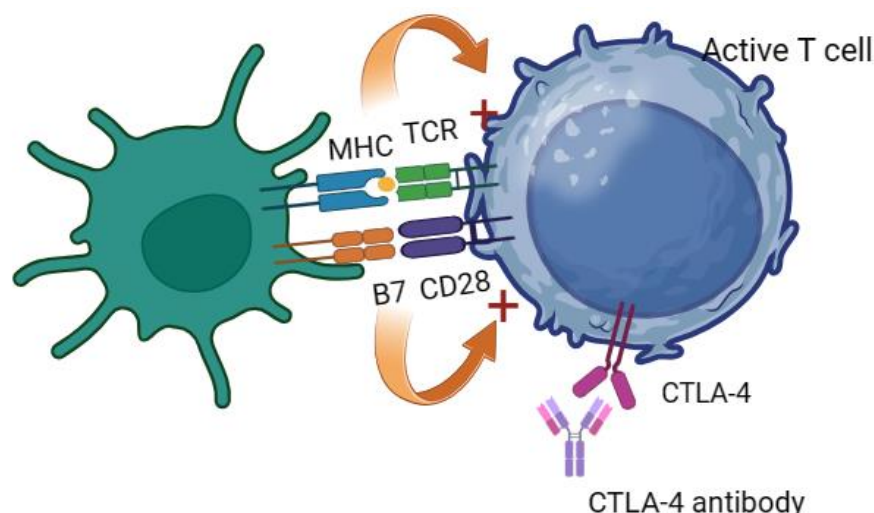


Figure 4. With the help of CTLA-4 antibody that has a higher affinity with CTLA-4 than B7, the CD28 can unite B7 and keep T cells active.

2.3. LAG-3

LAG-3 is a transmembrane protein that is characteristically expressed at T cells in activation, B cells, NK cells and also in plasmacytoid dendritic cells [13]. Four ligands of LAG-3 are reported in the past research which includes Galectin-3, major histocompatibility complex II (known as MHC II), along with fibrinogen-like protein 1 (known as FGL1), and also hepatic sinusoid endothelial cell lectin (known as LSECtin), with MHC II the major ligand [14]. Though it manifested that the inhibitory sound effect of LAG-3 on immune system is not aroused by the competitive binding between MHC II and CD4, it's sure that it helps tumor cells to escape from the immune system. LAG-3 is a important potential checkpoint in fighting against tumours and cancers, with many clinical researches and pre-clinical researches. A clinical phase 2-3 research uses relatlimab (known as a LAG-3 antibody) combined with Nivolumab (known as a PD-1 antibody) shows that with both relatlimab and nivolumab the median progression-free survival (PFS) was around 10.1 months (confidence level:95%; confidence interval (CI) :6.4 - 15.7) comparing with 4.6 months (confidence level:95%; CI:3.4 - 5.6) with nivolumab, meanwhile the rate of progression-free survival at 12 months was 47.7% (confidence level:95%; CI:41.8 - 53.2) with combination of relatlimab and nivolumab comparing with 36.0% (confidence level: 95%; CI:30.5 - 41.6) with nivolumab [15], which manifests a prosperous prospects of LAG-3 antibody unites with other immune checkpoint inhibitors. A clinical research testing the safety, efficacy and tolerability of a LAG-3 antibody (BMS-986016) combined or not combined with Nivolumab (PD-1 antibody) in treating the solid tumour (include gastric cancer) is on Phase 2 [16]. As we can see that there is still a big potential of designing Lag-3 inhibitors in treating gastric cancer, as LAG-3 is still a new discovered checkpoint even with not much medicine shooting on the market, with only Opdualag which is suitable for treating melanoma.

3. Treatment of Gastric cancer

For individuals with early-stage gastric cancer, the primary curative approach involves resection surgery, often accompanied by adjuvant chemotherapy (radiotherapy typically yields minimal benefits), resulting in a commendable five-year survival rate of over 90% [17]. However, the challenge lies in the atypical symptoms associated with gastric cancer, rendering early detection and diagnosis a formidable task. Consequently, the majority of patients are diagnosed at an advanced stage of the disease.

For patients diagnosed with advanced gastric cancer, treatment typically involves a combination of chemotherapy, targeted therapy, and immunotherapy, with a focus on inhibiting PD-1. In cases where chemotherapy alone is employed, using a platinum and fluoropyrimidine doublet regimen, the median

survival period is less than one year [18]. Targeted therapy with HER2 antibodies is limited to individuals with positive HER2 antigen tests. Notably, in a recent clinical phase 2 trial which involve advanced patients with HER-2 positive tumours, treatment with Trastuzumab Deruxtecan, an HER2 antibody, had a result of the median survival to be 12.5 months, as opposed to 8.4 months for those receiving chemotherapy [19]. Although this demonstrates a modest improvement in survival, it still falls short of reaching the two-year mark. In cases where PD-1 inhibitors are used as standalone therapy, they are generally well-tolerated, but their effectiveness tends to diminish in less than a year, largely due to the intricate tumor microenvironment [20].

Consequently, there is an urgent need for the development of new agents that target novel sites, particularly in the realm of immunotherapy, to extend a lifeline to patients battling advanced and metastatic gastric cancer. Furthermore, the demand for combination therapies, instead of relying solely on a single approach, is substantial. This approach is vital in countering the ability of tumor to develop tolerance, ultimately leading to an enhancement in the median survival rate.

4. Immunotherapy combined with other treatments

A clinical research for phase II used a designed TMEscore R package to provide tumor microenvironment statistics aiding the immunotherapy, and with a positive result [21]. In another Phase II research, patients who used zolbetuximab + EOX had a significant enhance in PFS with a hazard ratio (HR) to be 0.44 and the 95% CI from 0.29 to 0.67 ($P < 0.0005$) and also an ascend in OS with HR to be 0.55 and 95% CI from 0.39 to 0.77 ($P < 0.0005$) comparing with the patients who used EXO alone, which shows a prospect of combined therapy of first-line EOX with zolbetuximab, which is an immune checkpoint inhibitors [22]. A research showed the feasibility of a neoadjuvant unity of Nivolumab and ipilimumab neoadjuvant with ideal toxicity as well with a super high Polymerase Chain Reaction (PCR) rate (an indicator that manifests the growth speed of a tumour) in treating a dMMR/MSI-H gastric carcinoma/GEJ adenocarcinoma patient [23]. Another research combined camrelizumab (an immune checkpoint inhibitor), apatinib (an anti-angiogenesis agent) with S-1 ± oxaliplatin (a chemotherapy adjuvant drug) in treating cT4a/bN⁺ gastric carcinoma and resulted in a CPR of 15.8%, MPR of 26.3%, with highly satisfying safety and relatively high feasibility, which showed a good prospect of treating the MSI-H along with PD-L1 positive patients [24]. For first line therapy, patients with HER2-negative gastric/gastroesophageal junction cancer are faced a median overall survival for less than one years. Thus, a Chinese team is combining nivolumab and chemotherapy to treat patients in that situation. Compared with chemotherapy only, the united therapy of nivolumab together with chemotherapy had a successful increase in median overall survival (OS) (together 14.3 vs alone 10.2 months), median PFS (together 8.3 vs alone 5.6 months), ORR (together 66% vs alone 45%) and median DOR (together 12.2 vs alone 5.6 months), while the safety of such treatment was qualified [25]. Agent SHR-1701 is an antibody against PD-1 and TGF- β , which was in the first phase clinical test for advanced solid tumor (especially gastric cancer). The research tested the best dose for the second phase research (30mg/kg every three weeks, which with the highest ORR), with an ORR of 20% and 12-month overall survival rate of 54.5% [26]. It shows an positive efficacy and a standard toxicity and with a considerable prospect. Another research looked into a recent highlight pathway-STING pathway. Different from inhibiting the immune checkpoints to keep the T cells active, this pathway is in charge of innate immune pathway which can initiating the antitumor immunity [27]. A STING agonist (MSA-2) was made into low polarity pro-drug and was encapsulated by liposomes, which was intravenous injected into the tumor mice. The unity of PD-1 inhibitor with this STING agonist eradicated completely the tumors of mice compared with treating with each agent separately [28]. It shows a broad prospect in combining STING and immune checkpoint pathway together in the future clinical research and also the first line research, which can mobilize more immune cells and also more activated T cells in the tumor microenvironment.

5. Conclusion

Indeed, as indicated above, there are currently only a limited number of FDA-approved immune checkpoint inhibitors for gastric cancer, with only two agents targeting PD-1 having received approval.

However, there are numerous inhibitors in various phases of clinical testing. Some hospitals are actively investigating combinations of immunotherapy assisted with chemotherapy for the first-line treatment in dealing with the advanced gastric carcinoma. It is anticipated that more novel immune checkpoint inhibitors will be introduced to provide additional treatment options, particularly benefiting HER-2 negative advanced gastric carcinoma patients.

Moreover, the combination of various immune checkpoint inhibitors that target different immune checkpoints, along with the unite of immune checkpoint inhibitors with anti-angiogenic agents, chemotherapy, targeted antibody therapies, and even STING agonists, holds substantial promise as a therapeutic approach for advanced gastric cancers. These strategies aim to enhance the median survival rate of patients and represent significant avenues of research and development in the field.

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The prospects of targeting ferroptosis in cancer therapy

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Abstract. Ferroptosis represents a unique form of cell demise within the cellular framework, characterized by its reliance on intracellular iron levels, distinguishing it from apoptosis, necrosis, and autophagy. Emerging research indicates ferroptosis can restrict tumor growth, revealing potential new avenues for novel cancer treatments. The ability of ferroptosis to impede cancer cell proliferation highlights its promise as an innovative anti-tumor strategy. The fate of the cell is ultimately determined by the cellular machinery that governs ferroptosis. This central regulatory hub meticulously assesses the diverse pro-survival and pro-death cues it receives from subcellular organelles before making the determination of whether to initiate ferroptosis. This summarizes current knowledge about the regulatory mechanisms of ferroptosis, the crosstalk between apoptosis and ferroptosis, and the relationship between ferroptosis and specific organelles. Finally, some examples of drugs related to this topic are also discussed.

Keywords: Ferroptosis, Apoptosis, Organelles, Drugs.

1. Introduction

Ferroptosis is a type of regulated cell death, distinct from apoptosis, that is initiated through iron-mediated phospholipid peroxidation [1]. Ferroptosis is a form of regulated cell death characterized by its dependence on iron and increased lipid peroxidation. It is triggered when levels of the enzyme GPX4, which protects against phospholipid hydroperoxidation, are depleted, when redox-active iron accumulates to excess, and when phospholipids containing polyunsaturated fatty acids (PUFAs) undergo oxidative modification. The interplay between these factors - iron accumulation, loss of GPX4 activity, and peroxidation of PUFA-containing phospholipids - induces ferroptosis [2]. The cellular processes that regulate ferroptosis integrate a variety of signals, encompassing both pro-survival and pro-death cues from subcellular organelles, to ultimately decide whether to activate this lethal pathway or not [3]. Targeted cell death is a prevalent strategy in cancer treatment. Given that ferroptosis inducers have the potential to specifically target cancer cells, incorporating ferroptosis-inducing medications can enhance the anti-tumor effectiveness of these drugs [4]. In current cancer treatment, clinical compounds induce ferroptosis mainly by suppressing System Xc⁻ and GPX4. Additionally, some natural products can stimulate ferroptosis specifically in cancer cells, underscoring their importance. The combined effects of synthetic inhibitors and natural inducers that target these two key proteins represent the major mechanisms for exploiting ferroptosis therapeutically.

In this review, this summarizes our current comprehension of the regulatory factors that govern ferroptosis, the interactions involving ferroptosis and particular organelles, as well as the connections

between apoptosis and ferroptosis. In conclusion, several drug examples are associated with these mechanisms. It is anticipated that this review will contribute to an improved comprehension of ferroptosis and provide the groundwork for future investigations and the advancement of cancer therapies based on ferroptosis.

2. Ferroptosis

Ferroptosis is a specific type of regulated cell death that is distinct from apoptosis. It constitutes a novel form of cell demise with characteristic features that differentiate it from other modes of programmed cell death. The properties that define ferroptosis make it a unique cell death pathway separate from apoptotic and other cell death programs. It is primarily marked by its dependence on iron-induced phospholipid peroxidation [1]. Ferroptosis can be characterized by two main pathways: the extrinsic route, relying on transporters, and the intrinsic route, governed by enzymes [5]. Ferroptosis can take place in both cancer cells and normal cells; however, selectively inducing ferroptosis in tumors while safeguarding normal tissues remains a challenge. Ferroptosis assumes a pivotal role in regulating the initiation and evolution of various diseases, making it a focal point of research and a subject of keen interest for improving the management and prognosis of these related conditions (Figure 1).

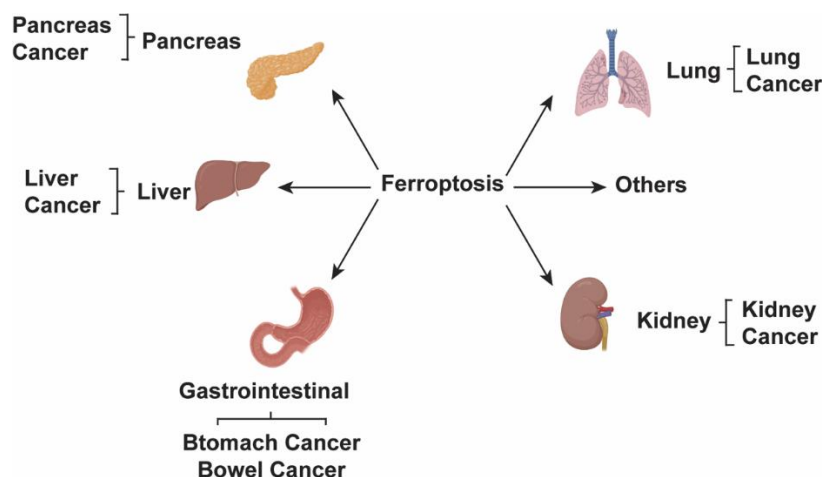


Figure 1. Ferroptosis has assumed significant roles in the pathogenesis of various systemic diseases.

2.1. The characteristics of ferroptotic cell death

Ferroptosis is a type of regulated cell death that relies on iron accumulation and elevated lipid peroxidation. The key features that define ferroptosis are its requirement for iron and accumulation of oxidized lipid damage. Ferroptosis is characterized by an impaired capacity to repair lipid peroxides. The dependency of ferroptosis on iron and lipid peroxidation arises from depleted levels of the lipid repair enzyme GPX4, accumulation of redox-active iron, and iron-mediated oxidation of phospholipids containing polyunsaturated fatty acids. The combination of deficient GPX4 activity, iron excess, and resulting phospholipid damage underlies the reduced lipid repair during ferroptosis [2]. Cells undergoing ferroptosis exhibit morphological characteristics that are unique compared to other modes of cell demise. Hallmarks of ferroptosis include smaller mitochondria with compacted membranes and disappearance of mitochondrial cristae. These distinct mitochondrial changes give ferroptotic cells a characteristic ultrastructural appearance that sets them apart. These mitochondrial changes give ferroptotic cells a distinctive appearance [6].

2.2. Methods to promote and prevent ferroptosis

Surplus iron is sequestered within ferritin and can be transported to lysosomes for degradation via the function of NCOA4, leading to the liberation of iron. This process, referred to as ferritinophagy, can

promote the initiation of ferroptosis [7]. The onset of ferroptosis can be averted by employing iron chelators, while introducing exogenous iron intensifies ferroptosis [8].

3. The relationship between ferroptosis and specific cell organelles

The cellular apparatus orchestrating ferroptosis assimilates diverse signals from subcellular organelles, encompassing both pro-survival and pro-death cues, before determining whether to initiate the lethal pathway or not [3].

3.1. Ferroptosis with endoplasmic reticulum

The endoplasmic reticulum (ER) exhibits a high degree of sensitivity to alterations in the intracellular milieu, including changes in pH and viscosity. Even slight variations can elicit stress responses within the ER [9]. Ferroptosis is an exceptional, non-apoptotic pathway of cell demise linked to diseases connected with endoplasmic reticulum stress [10]. Viscosity, as a pivotal physicochemical factor, is intricately connected to diffusion-driven mechanisms, exerts significant influence on the control of various biological processes, and serves as a vital gauge for discerning numerous subcellular biological activities [11]. During the ferroptosis process, an iron-dependent Fenton reaction takes place, generating lipid radicals. Subsequently, unsaturated lipids undergo conversion into lipid peroxides through radical-initiated reactions.

3.2. Ferroptosis with mitochondria

Mitochondria assume a crucial role in the context of ferroptosis initiated by cysteine deficiency. However, mitochondrial abnormalities are not critical for ferroptosis induced by inhibition of glutathione peroxidase-4 (GPX4), the terminal effector in the ferroptotic pathway. Iron can be transported into mitochondria by SLC25A37 and SLC25A28 for heme/Fe-S cluster biosynthesis or stored in mitochondrial ferritin. But mitochondrial dysfunction is not an essential feature of GPX4 inhibition-induced ferroptosis. Heme, when catalyzed by HMOX1, decomposes into Fe^{2+} and plays a critical role as a cofactor in the electron transport chain (ETC), thereby supporting ferroptosis. The suppression of proteins engaged in iron-sulfur cluster formation, including NFS1, FXN, and ISCU, can intensify ferroptosis by provoking an iron-deprivation response mediated by IREB2. On the other hand, iron-sulfur (Fe-S) proteins CSD1 and CSD2 mitigate ferroptosis by lowering intracellular iron levels [3]. Mechanistically, cysteine deficiency leads to hyperpolarization of the mitochondrial membrane potential and accumulation of lipid peroxides. Inhibition of either the mitochondrial tricarboxylic acid (TCA) cycle or electron transport chain (ETC) can normalize the hyperpolarized mitochondrial membrane potential, decrease lipid peroxide buildup, and suppress ferroptosis. The mitochondrial hyperpolarization and lipid peroxidation induced by cysteine deprivation contribute to ferroptosis, and targeting mitochondrial metabolism can reduce these effects and ferroptotic cell death (Figure 2) [12].

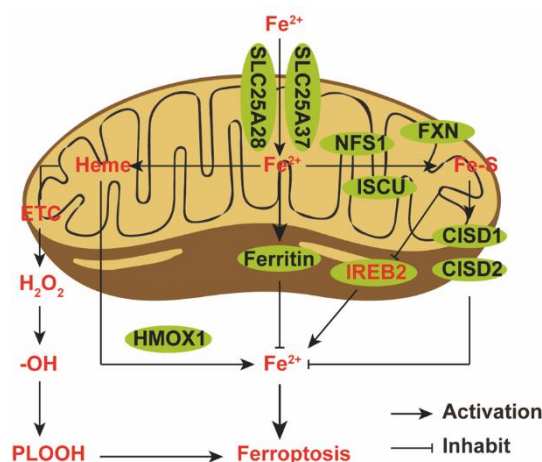


Figure 2. Mitochondrial iron.

3.3. *Ferroptosis with Golgi*

Various compounds that modulate ferroptosis exhibit protective properties on Golgi structure and function when cells are exposed to the Golgi stressors mentioned earlier. Ferroptosis inhibitors safeguard cells from Golgi dispersion and also impede protein secretion triggered by multiple Golgi stress agents [13].

4. **The cellular pathways linking ferroptosis and apoptosis**

Ferroptosis is a newly characterized form of regulated cell death uniquely dependent on iron-mediated lipid peroxidation. Its distinguishing characteristic is the triggering of cell death through iron-catalyzed oxidation of lipid molecules. A defining feature of ferroptosis is the accumulation of lipid peroxides and destructive reactive oxygen species. This iron-catalyzed lipid peroxidation is what sets ferroptosis apart as a distinct mode of programmed cell death. The presence of these oxidized lipids and free radicals is a distinguishing characteristic. Extensive research has been conducted on ferroptosis in recent years. Initially, ferroptosis and apoptosis were thought to be mutually exclusive cell death pathways. However, recent evidence suggests that there is significant interplay between ferroptosis and apoptosis. In specific cellular scenarios, these two cellular demise pathways engage in a finely tuned interplay. Multiple subcellular locations and signaling molecules play a role in governing both ferroptosis and apoptosis. These sites and molecules act as modules or switches, allowing the cell to decide which death pathway to activate. Overall, the relationship between ferroptosis and apoptosis is complex and context-dependent. A nuanced understanding of how cells navigate between these two death programs will provide insight into both normal physiology and disease pathology [14].

4.1. *The pathways of ferroptosis*

Ferroptosis is initiated by an excess of reactive oxygen species (ROS) generated as a result of an unbalanced redox reaction, stemming from reduced glutathione synthesis and the inactivity of the enzyme glutathione peroxidase 4 (GPX4) [15]. This deadly process is characterized by buildup of reactive oxygen species in lipids resulting from iron excess, as well as reduction of polyunsaturated fatty acids in the cell membrane. Cancer cells exhibiting hyperactivation of the RAS-RAF-MEK pathway or p53 expression are often more susceptible to this form of cell death. The interaction between iron, lipid peroxides, and membrane polyunsaturated fatty acids fuels the execution of this lethal cascade [16].

4.2. *The pathways of apoptosis*

Apoptosis, the regulated cell death process, involves two main pathways, namely, the extrinsic and intrinsic pathways, along with an additional pathway involving perforin and granzymes. Each of these pathways requires specific triggering signals to initiate a series of energy-dependent molecular events. Within each pathway, there are distinct initiator caspases (8, 9, 10) responsible for initiating the activation of the executioner caspase-3 [17].

4.3. *Interactions between ferroptosis and apoptosis*

I will make comparisons and highlight differences between ferroptosis and other forms of regulated cell death, such as apoptosis. Examining the similarities and differences between ferroptosis and other regulated cell death pathways provides insight into this process [18]. Starvation, as well as the administration of gallic acid and exposure to irradiation, lead to an increase in reactive oxygen species (ROS). Subsequently, ROS induces both apoptosis and ferroptosis, particularly through lipid ROS. TRIP-Br1 reduces ROS levels, with its inhibition being facilitated by the PI3K/Akt pathway. PUFAs instigate ferroptosis by generating lipid ROS, and simultaneously, they activate apoptosis through the PPAR γ /SDC-1 pathway. GPX4 and glutathione (GSH) counteract lipid-derived reactive oxygen species (ROS). However, these protective mechanisms are thwarted by compounds like danshen (DT) and PEITC, which also have the capacity to induce apoptosis. Additionally, the tumor suppressor protein p53 serves a versatile function by promoting ferroptosis through the suppression of SLC7A11 and by

initiating apoptosis through the regulation of pivotal genes, including BAX, APAF-1, PUMA, p53AIP1, PIDD, and NOXA (Figure 3) [18].

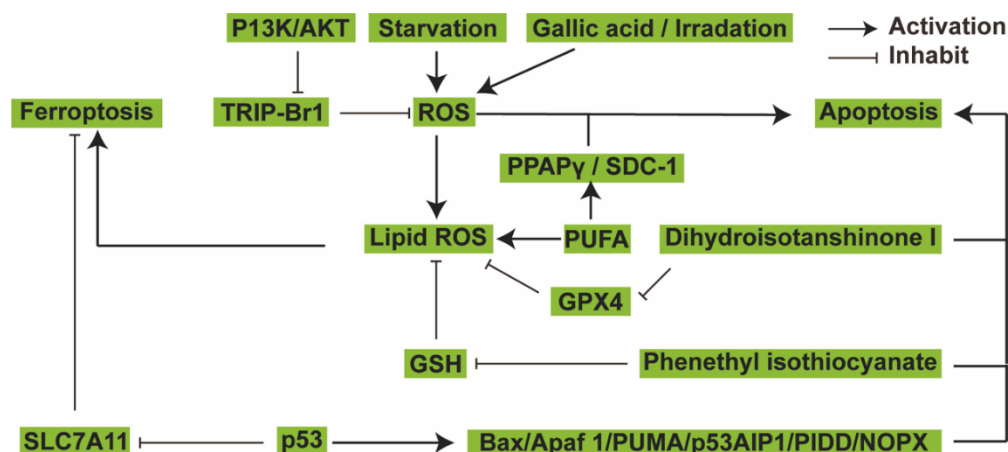


Figure 3. The crosstalk between ferroptosis and apoptosis.

5. Drugs that induce ferroptosis in cells

Stimulating cell death pathways is a prevalent strategy in cancer therapy. Because ferroptosis-triggering compounds can selectively target cancer cells, combining them with current therapies may enhance anti-tumor effects. The cancer cell-specific lethality of ferroptosis inducers makes them promising agents to integrate into existing cytotoxic regimens [4]. Current clinical ferroptosis inducers used for cancer treatment mainly work by inhibiting System Xc⁻ and GPX4 function. The primary anti-tumor mechanism of these compounds involves suppression of these two key proteins. System Xc⁻ is an amino acid transporter that mediates the exchange of extracellular cystine for intracellular glutamate across the plasma membrane. This antiporter activity is essential for the import of cystine and export of glutamate between the outside and inside of the cell (Figure 4) [19]. Moreover, specific natural compounds have a substantial impact on provoking ferroptosis in cancer cells (Table 1).

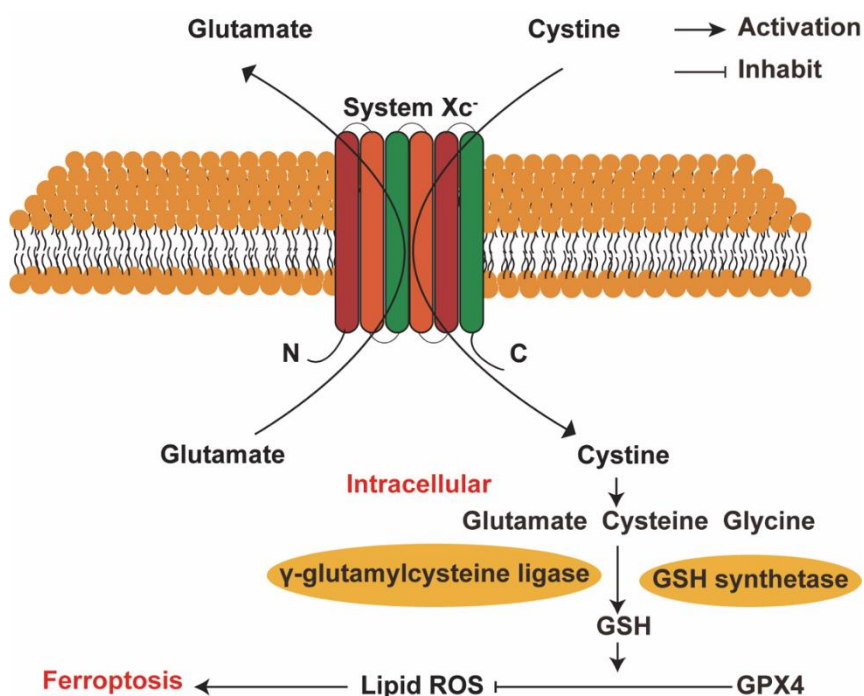


Figure 4. System Xc⁻ Process of transferring amino acid.

Table 1. Preclinical drugs associated with ferroptosis.

Ferroptosis inducer	Clinical/preclinical findings	Mechanisms of action	References
Erastin	Gastric cancer treatment	Inhibit System Xc ⁻ , GSH depletion	[4, 20]
Sulfasalazine	Ovarian cancer and breast cancer treatment	Inhibit System Xc ⁻ , GSH depletion	[21]
Sorafenib	Hepatocellular carcinoma and lung adenocarcinoma	Inhibit System Xc ⁻ , GSH depletion	[22]
RSL3	Ovarian cancer	GPX4 inactivation	[23, 24]
ML162	Induced head and neck cancer cell death	Inhibit GPX4, induces lipid peroxidation	[25]
ML210	Mesenchymal-high cancers	Covalently modified selenocysteine of GPX4	[25]
FIN56	Breast carcinoma cells	Deplete CoQ10, decreases GPX4 level	[5, 26]
Buthionine sulfoximine (BSO)	Pancreatic cancer	Decreases GSH and GPX4 level	[27]
Dihydroartemisinin	Glioma cells	Induces ROS, decreases GSH and GPX4 level	[28, 29]
Siramesine	Breast cancer	Increasing the levels of transferrin and ROS	[30, 31]

5.1. Inhibition of the System Xc⁻ activity

System Xc⁻ works mainly by swapping intracellular glutamate for extracellular cystine. It moves glutamate out of the cell and brings cystine in from the external environment. This exchange of internal glutamate for external cystine represents the key functional activity of system Xc⁻. The imported cystine can then be utilized to generate glutathione within the cell. When ferroptosis inducers inhibit System Xc⁻, the synthesis of glutathione diminishes. Consequently, GPX4 becomes unable to utilize glutathione for the reduction of lipid peroxides, ultimately leading to cell ferroptosis [32]. The primary regulation of System Xc⁻ activity is governed by SLC7A11. A reduction in the expression of SLC7A11 results in decreased System Xc⁻ activity, leading to oxidative stress-induced ferroptosis. Conversely, the upregulation of SLC7A11 enhances the cellular resilience to ferroptosis, a substantial factor contributing to drug resistance in cancer cells [33]. For examples, such drugs are Erastin, Sulfasalazine (SAS) and Sorafenib, etc.

5.1.1. Erastin. Erastin, a small molecule, can trigger ferroptotic cell death in cancer cells, demonstrating significant promise for cancer therapy. However, it is crucial to highlight that erastin-induced ferroptosis led to pathological changes in normal tissues in mouse models as well. This observation implies that the anti-tumor drug erastin may exhibit some level of toxicity to healthy tissues [20]. Erastin has the capability to both prevent and reverse the obstruction of VDAC (voltage-dependent anion channel) by cytoplasmic free tubulin, both in vivo and in vitro, thereby facilitating the opening of the voltage-dependent anion channel [4].

5.1.2. Sulfasalazine. Sulfasalazine (SAS) triggers ferroptosis and exhibits potential in tumor therapy. Glioma cells become more resistant to SAS-induced ferroptosis in low oxygen conditions. The SLC7A11 gene is critical for glioma cells to defend against ferroptosis under hypoxia. Testing the HIF-1 α inhibitor PX-478 together with SAS in mouse models revealed it can decrease SLC7A11 levels in tumors, potentiating SAS's anti-cancer activity. Hypoxic tumor environments limit ferroptosis sensitivity through SLC7A11 upregulation, and inhibiting HIF-1 α with PX-478 counters this resistance mechanism [21].

5.1.3. Sorafenib. Cells demonstrating resistance to sorafenib display a marked elevation in intracellular iron content and ROS. Significantly boosting the efficacy of sorafenib as an anti-tumor agent, the suppression of MT-1G expression induces ferroptosis, demonstrating its impact in both in vivo and in vitro settings. Likewise, decreasing expression of ABCC5 substantially improves the anti-cancer efficacy of sorafenib, enhancing its ability to trigger ferroptosis in both cell culture and animal models. Suppressing ABCC5 levels sensitizes tumor cells to sorafenib-induced ferroptosis, underscoring the importance of ABCC5 as a resistance factor that impedes ferroptosis [22].

5.2. Inhibition of the GPX4 activity

Some ferroptosis-stimulating agents act intrinsically to inhibit the antioxidant enzyme GPX4. These compounds promote ferroptosis by directly targeting and suppressing GPX4 activity through internal pathways within the cell [32]. For examples, such drugs are RSL3, ML162, ML210, FIN56 and Buthionine sulfoximine (BSO), etc.

5.2.1. RSL3. RSL3 induces ferroptosis by elevating reactive oxygen species and labile iron levels in cells. RSL3-treated colorectal cancer cells show increased transferrin expression but decreased GPX4, reflecting the iron dependence of this death process. RSL3 drives ferroptosis through iron accumulation combined with oxidative stress and suppression of the lipid antioxidant GPX4 [23]. The growth-inhibitory impact of RSL3 on glioma cells is linked to the disruption of glycolysis and the initiation of autophagy [24].

5.2.2. ML162 and ML210. In both the MDA-MB-231 breast cancer cell lines and Hec50 uterine serous carcinoma cells, the knockout of metadherin (MTDH) resulted in a marked decrease in their responsiveness to GPx4 inhibitors, specifically ML162 and ML210 [25].

5.2.3. FIN56. As a newly identified ferroptosis inducer, FIN56 displayed a marked anti-tumor impact, leading to lysosomal membrane permeabilization through a mechanism that relies on the transcription factor EB (TFEB) in glioma [26].

5.2.4. Buthionine sulfoximine. The influence of L-BSO was evaluated in an in vivo ovarian cancer model, leading to a substantial decrease in subcutaneous tumor size, as well as a reduction in GSH levels and a mitigation of peritoneal dissemination. However, the observed toxicity of L-BSO may limit its suitability for therapeutic use [27].

5.3. Other drugs induce ferroptosis

Dihydroartemisinin (DHA) can stimulate the lysosomal breakdown of ferritin, a process independent of autophagy. This leads to an augmentation of cellular free iron levels, making the cells more vulnerable to ferroptosis [34]. Co-administration of siramesine, a lysosome-destabilizing compound, and the dual tyrosine kinase inhibitor lapatinib synergistically induces cancer cell death in breast tumors. This synergistic lethal effect is achieved predominantly through the induction of ferroptosis. The concurrent lysosomal perturbation caused by siramesine and tyrosine kinase inhibition by lapatinib collaboratively activate ferroptotic cell death pathways in breast cancer cells. The synergistic interplay between these two agents potently promotes ferroptotic cell demise [35]. Inhibition of mitochondrial complex I activity by BAY 87-2243 triggers apoptotic cell death in BRAFV600E mutant melanoma cell lines and impedes melanoma tumor growth in vivo [36].

5.3.1. Dihydroartemisinin. The lethal impact of dihydroartemisinin (DHA) on glioblastoma encompasses several facets, including cytotoxicity, cell cycle arrest, and the suppression of invasion. Artemisinin and its derivatives trigger ferroptosis, resulting in the suppression of head and neck carcinoma and fibrosarcoma [28]. Exposure to DHA detrimentally affects embryos in both rodent and

primate models in vitro, primarily through mechanisms that influence cytoskeletal dynamics, oxidative stress, calcium balance, and apoptosis [29].

5.3.2. Siramesine. Siramesine was discovered to have a direct destabilizing effect on the lysosomal membrane. This disruption causes a disturbance in lysosomal operation, ultimately culminating in membrane permeability and the discharge of cathepsins into the cytosol. Consequently, this process results in cell death mediated by cathepsins [30]. Research has shown that Siramesine works synergistically with other agents to elicit ferroptosis, a form of regulated cell death, in breast cancer cells. The mechanism involves Siramesine increasing transferrin levels and promoting ROS accumulation, leading to iron-dependent lipid peroxidation and eventual cell death [31].

6. Potential therapy of cancer through ferroptosis

Immunotherapy employing immune checkpoint inhibitors has marked a significant advancement in oncology and demonstrated considerable effectiveness against various malignancies. Nonetheless, the persistence of drug resistance poses a formidable challenge. The factors contributing to resistance to immunotherapy encompass both inherent characteristics within tumor cells and external elements associated with the tumor microenvironment [37]. Tumor cells' intrinsic factors, including their genetic or phenotypic traits, can impede the infiltration or proper functioning of immune cells within the tumor microenvironment. In addition to intrinsic factors, extrinsic elements stemming from the tumor microenvironment, excluding the tumor cells themselves, can also suppress anti-tumor immunity [37].

6.1. Tumor-cell-intrinsic mechanisms

Multiple intrinsic properties of tumor cells that confer resistance to immunotherapy have been discovered. These include activation of MAPK signaling, loss of PTEN expression, activation of WNT/ β -catenin signaling, defects in interferon-gamma signaling, and downregulation of tumor antigen expression [37]. Inducing ferroptosis, a controlled cell death process, within cancer cells has the potential to generate an immune response akin to vaccination, enhancing anti-tumor immunity and potentially surmounting resistance to immunotherapy [1].

6.2. Tumor-cell-extrinsic mechanisms

Aside from intrinsic tumor cell factors, immunosuppressive cells residing in the tumor microenvironment (TME) can also facilitate resistance to immunotherapy. These immunosuppressive cells encompass regulatory T cells (Tregs) and tumor-associated macrophages (TAMs), both capable of suppressing anti-tumor immunity. The presence of Tregs and TAMs can restrain immune responses against tumors. The presence of these cells in the TME creates an immunosuppressive milieu that enables tumors to evade immune attack [37]. Inducing ferroptosis within the TME could potentially serve as an extrinsic strategy to overcome immunotherapy resistance by eliminating immunosuppressive cells like Tregs and TAMs. Triggering regulated cell death through ferroptosis may therefore deplete these inhibitory immune populations in the TME and enhance anti-tumor immunity.

7. Conclusion

Ferroptosis is a regulated mode of cell death distinct from apoptosis, primarily driven by iron-mediated peroxidation of phospholipids. It is characterized by buildup of ROS and excessive formation of lipid peroxides. The defining features of ferroptosis are iron-catalyzed lipid oxidation leading to ROS accumulation and lipid peroxide overproduction, separating it as a unique form of programmed cell death apart from apoptosis [1]. Regulating cell demise pathways is a traditional tactic in oncology, and ferroptosis specifically has gained recognition as an important component of anti-cancer therapies in modern times. The cellular machinery responsible for regulating ferroptosis assimilates a spectrum of pro-survival and pro-death cues from subcellular organelles, ultimately reaching a verdict on whether to activate this lethal pathway or not [3]. Numerous investigations have showcased the clinical promise of specific ferroptosis inducers. These findings have been pivotal in propelling novel therapeutic

approaches for cancer treatment forward. These approaches leverage ferroptosis, a type of iron-reliant programmed cell death, to specifically target and eradicate cancer cells. By elucidating and controlling the mechanisms of ferroptosis, novel treatments seek to activate this cell death process selectively in tumor cells as an anti-cancer tactic. The goal is to exploit the ferroptotic machinery to induce cancer cell demise in a precise manner [4].

In this review, this summarizes the current comprehension of the regulatory mechanisms that govern ferroptosis, the interplay between apoptosis and ferroptosis, as well as the association between ferroptosis and specific cellular organelles. Finally, some examples of drugs related to this. While some inducers of ferroptosis are currently being explored in clinical trials, clinical data remains insufficient at this time. There are presently no approved drugs leveraging ferroptotic cell death mechanisms available on the market, and significant work remains to translate our understanding of iron-dependent cell death pathways into practical clinical applications.

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Progress of related drugs and new drugs for immunotherapy of triple-negative breast cancer

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Abstract. Triple-negative breast cancer (TNBC) stands as the most aggressive kind of breast cancer, characterized by a terrible prognosis. TNBC is more prevalent in patients under 40 years old and in those with *BRCA1* mutations. Treatment for TNBC typically involves chemotherapy, radiation therapy, targeted therapy, immunotherapy, and other approaches. However, the discovery of new therapeutic interventions is hampered because of the absence of precisely defined targets, making immunotherapy a prospective option for giving greater benefits to TNBC patients. Based on numerous clinical studies, we explore the research development of various immune checkpoint inhibitors in the therapies of TNBC in this review. Programmed death-1 (PD-1) inhibitors, programmed death-ligand 1 (PD-L1) inhibitors, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors, antibody-drug conjugates, and PARP inhibitors are among these inhibitors. Additionally, we briefly introduce novel immunotherapies and combination therapies.

Keywords: drugs for triple-negative breast cancer, immunotherapy, novel immunotherapy approaches, combination therapy

1. Introduction

TNBC is breast cancer that expresses neither progesterone receptor (PR), estrogen receptor (ER), nor human epidermal growth factor receptor-2 (HER-2). One in six occurrences of breast cancer are caused by it, with a 5-year recurrence and metastasis rate of 15% and a survival rate of approximately 15% after 5 years. Compared to other subtypes, TNBC has a worse prognosis and a higher chance of recurrence.

In TNBC immunotherapy, the immune system's activation is critical. The immune system can resist foreign or internal bacteria, viruses, and other harmful ingredients, and it prevents tumor growth and reduces the possibility of metastasis by monitoring, recognizing and destroying cancer cells in real time. To protect healthy cells, the immune system uses immunological checkpoints to perform self-control [1]. This method is based on the recognition and binding of T cell receptors (TCRs) to antigens contained in major histocompatibility complexes (MHCs) on the surface of antigen presenting cells (APCs). CTLA-4, PD-1, and PD-L1 are all related to T cell immune response inhibition.

With 288 amino acids, PD-1 is a transmembrane protein. The protein's structure contains extracellular IgV domains, a transmembrane region, and an intracellular tail region. PD-1 inhibits TCR signaling, as evidenced by the two phosphorylation sites found in the immunoreceptor tyrosine-based switch and inhibitory motif in the intracellular tail region. The *PDCD1* gene encodes the human PD-1 protein. One of the ligands of PD-1, PD-L1, is a protein that the human *CD274* gene encodes. This protein is also a transmembrane, weighing 40 kDa in molecular weight. T cell proliferation is inhibited by signals transmitted by the combination of PD-L1 and PD-1 on T cells. A protein receptor which functions as an immunological checkpoint and suppresses immune responses is called CTLA-4, or CD152. The human *CTLA-4* gene encodes the CTLA-4 protein. This protein includes an extracellular V domain (VL), a transmembrane domain, and a cytoplasmic tail region.

Immunotherapy has reflected promising results in many diseases of gastric cancer, kidney cancer, melanoma and so on. In TNBC, monoclonal antibodies, alone or in conjunction with various treatments involving chemotherapy, radiation, neoadjuvant therapy, and targeted therapy, seem promising in both beginning and later stages of the disease.

2. The difficulty of treating TNBC in comparison to other types of breast cancer

TNBC is distinguished by early onset, high malignancy and invasiveness, poor prognosis, and high recurrence rate. ER, PR and HER-2 are important anti-cancer targets for other breast cancers. The negative expression of ER, PR and HER-2 means that these three receptors are not expressed on the surface of breast cancer cells, or their expression levels are low, which will limit endocrine therapy, targeted therapy, hormone therapy, etc. Common traditional treatments include chemotherapy and cytotoxic therapy. Although chemotherapy can reduce the risk of recurrence of TNBC, the overall survival rate has not significantly improved; cytotoxic therapy can cause severe cardiac toxicity and damage the gastrointestinal tract. Therefore, in recent years, with the continuous enrichment of treatment methods, immunotherapy, including combination with other treatments, has shown great potential to have relatively greater survival benefits for TNBC patients.

3. Overview of TNBC immunotherapy

According to current clinical trials and drug research, TNBC immunotherapy mainly includes the following: checkpoint inhibitor therapy, antibody-drug conjugate therapy, PARP inhibitor therapy, and some other novel immunotherapy methods (Figure 1).

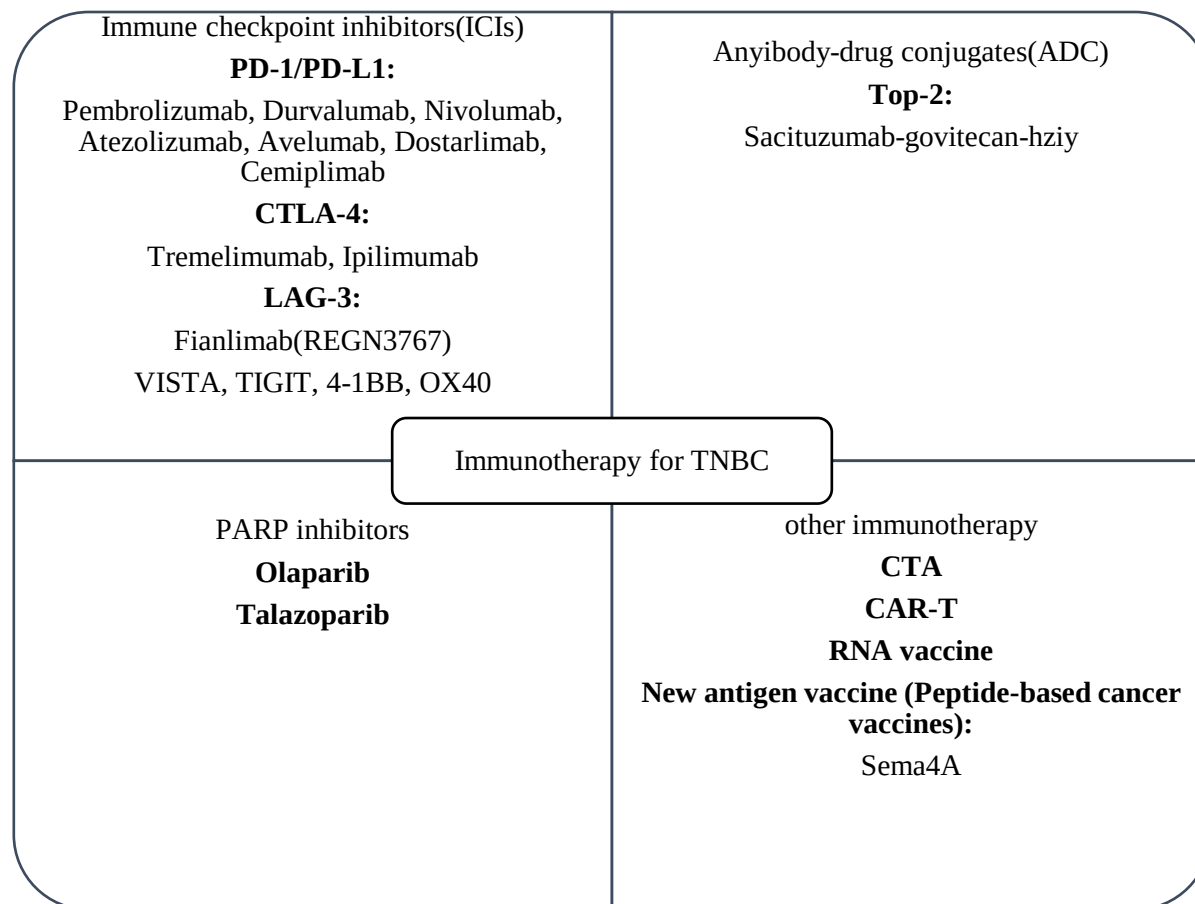


Figure 1. Common therapies for TNBC immunotherapy.

3.1. Immune Therapy with Checkpoint Inhibitors

Currently, ongoing clinical studies with immune checkpoint inhibitors can be broadly categorized into two types: monotherapy and combination therapy with other drugs. The latter is frequently used for treating locally progressed or metastatic triple-negative breast cancer (mTNBC). In clinical practice, most immune drugs are monoclonal antibodies targeting PD-1 and PD-L1, as well as some commonly used monoclonal antibodies against *CTLA-4*, *Lymphocyte activation gene-3 (LAG-3)*, etc. Chemotherapy combined with immune therapy has been proven to possess safety and tolerance in other malignancies. Pure chemotherapy may generate new antigens and disrupt tumor immune evasion mechanisms [2]. Some cancers also have a low response rate to single-dose immune checkpoint blocking, so chemoimmunotherapy will have a better effect in clinical treatment and even have a certain synergistic effect.

3.1.1. PD-1 Inhibitors. Pembrolizumab refers to a humanized monoclonal immunoglobulin G4 antibody which connects to the PD-1 and suppresses its interaction with PD-L1 and PD-L2 [3,4]. The use of pembrolizumab is one of the methods for clinical treatment of mTNBC. In a study, combined with pembrolizumab (immunotherapy) and eribulin (chemotherapy) was effective in treating mTNBC. The study included 167 individuals, with 7 in stage Ib and 160 in stage II. Group 1 had 66 patients that had never been treated before. The objective response rate and overall response rate (ORR) were both 25.8%; Group 2 included 101 patients who had already had therapy, and the ORR was counted as 21.8%. Patients with PD-L1⁺ tumors had a statistically greater ORR (combined positive score 1) than patients with PD-L1⁻ tumors [5]. Another phase II clinical trial with pembrolizumab combination therapy, in comparison to a historical placebo group, patients received an intravenous injection of 200mg paclitaxel

and oral therapy of 1000mg/m² capecitabine every two days in the first two weeks of a twenty-one-day regimen. The main outcome was median progression-free survival (mPFS), while other outcomes were ORR, safety, and tolerability analysis, all of which showed significant improvement in the results. Compared with a historical control group treated with capecitabine alone, it was found that 80% of the subjects showed significant improvement in mPFS after adding paclitaxel for 2 months [6]. In the treatment of TNBC, the use of Pembrolizumab can improve event-free survival rate, but the prognosis still needs to be optimized to reduce the occurrence of adverse events (AEs) such as pneumonia, rash, and fatigue.

Nivolumab refers to a monoclonal antibody which targets the immunological checkpoint receptor PD-1 on the surface of T cells. Nab-paclitaxel has shown significant efficacy in other solid tumors, but clinical research on TNBC is still limited and currently in the in vitro research stage [7].

3.1.2. Inhibitors targeting PD-L1. Durvalumab is a human immunoglobulin G1κ (IgG1κ) monoclonal antibody which can inhibit the cooperation of PD-1 and PD-L1. In a clinical trial of durvalumab together with olaparib and paclitaxel, 73 participants randomly received standard paclitaxel neoadjuvant chemotherapy (DOP), and 299 participants received standard care (paclitaxel) control group. The combined efficacy of durvalumab and Olaparib raised the expected pathological complete response (pCR) rate from 20% to 37% (exceeding the control), from 27% to 47% in TNBC. AEs included thyroid dysfunction, fatigue, pneumonia, etc. [8].

Atezolizumab is an IgG1 monoclonal antibody which has been humanized and designed to be anti-PD-L1. In terms of efficacy, atezolizumab combined with albumin-bound paclitaxel can improve the treatment effect of TNBC, although this method significantly prolongs overall survival (OS) effect, this combination therapy also significantly alters PFS and ORR. However, compared with the use of albumin-bound paclitaxel alone, this combination produces greater toxicity [9]. There were 68 patients in a phase 2b clinical trial using atezolizumab coupled with anthracycline drugs for the treatment of mTNBC [10]. In the first group of 40 patients who got pegylated liposomal doxorubicin and cyclophosphamide at a low intensity, 18% experienced AEs, while the second group of 28 patients who received placebo, 7% experienced AEs [10]. In this study, the PFS of Group 1 improved after several months. After 15 months, the PFS of Group 1 further improved, with a rate of 14.7%, while the progression-free rate of Group 2 was 0% [10]. The combination therapy of atezolizumab monoclonal antibody had a higher incidence of AEs compared to monotherapy, mainly involving dermatology, gastrointestinal tract, and hematology, such as lymphadenopathy and subcutaneous nodules in the limbs [11].

Avelumab refers to an immune checkpoint inhibitor which blocks the PD-1 and PD-L1 pathway but doesn't influence their relationship against human IgG1 monoclonal antibody which targets PD-L1. In a phase 1b study of JAVELIN solid tumor, there were 168 metastatic breast cancer (MBC) patients, comprising 58 TNBC patients, received avelumab treatment for 2-50 weeks. 13.7% of all experienced grade ≥3 treatment-related AEs, involving two cases of treatment-related deaths. The main AEs were fatigue, nausea, and hypothyroidism. The confirmed ORR for TNBC patients was 3.0% and 5.2%. PD-L1⁺ tumor-infiltrating immune cells demonstrated a better ORR than PD-L1⁻ tumor-infiltrating immune cells in the total sample (16.70% vs 1.60%) and the TNBC subset (22.20% vs 2.60%) [12]. As a result, in a subgroup of TNBC patients, avelumab demonstrated adequate safety and clinical efficacy.

3.1.3. Inhibitors targeting CTLA-4. One of the earliest immune checkpoint medications to target the CTLA-4 pathway was tremelimumab. This IgG2 monoclonal antibody inhibits the interactions of the antigen-presenting cell ligands B7-1 (CD80), B7-2 (CD86), and CTLA-4 receptor generated on activated T cells, leading to an anti-tumor effect. There were 18 evaluable patients in a single-arm pilot study, 11 ER⁺ and 7 TNBC, and only three patients have the response (ORR=17%), hence the project didn't progress to Phase 2. It can be seen that pertuzumab has a better clinical benefit rate in TNBC, but attention should also be paid to the occurrence of AEs such as rash and hepatitis [13].

Ipilimumab is a CTLA-4 pathway antagonist. It is a human IgG1 monoclonal antibody which blocks to the CTLA-4 receptor on activated T cells. Despite multiple clinical trials for the therapy of tumors that are solid, there has been minimal research on the efficacy and toxicity of TNBC. A total of 19 individuals were included in a clinical trial testing ipilimumab in breast cancer patients, with 12 receiving ipilimumab treatment. Among these 12 patients, 6 received ipilimumab monotherapy, and the remaining 6 patients received cryoablation treatment in addition to ipilimumab monotherapy. Following combination immunotherapy, there was a rise in T helper type 1 cytokines in the tumor. Therefore, the combined use of ipilimumab and cryoablation may be beneficial for the therapy of breast cancer. This medication may cause diarrhea, constipation, and other side effects [14].

3.1.4. Other monoclonal antibody drugs and other targets. Dostarlimab is a PD-1 receptor antagonist presently being tested in clinical trials for the therapy of many different malignancies. Cemiplimab is a monoclonal IgG1 antibody which inhibits the PD-1 pathway. LAG-3 is a novel immunological therapy target that, in conjunction with PD-1, suppresses T cell activity [15]. Fianlimab (REGN3767) is another LAG-3 targeting antibody being tested as a neoadjuvant therapy with cemiplimab. Other immunotherapy techniques targeting checkpoint inhibitors are being studied, including VISTA [16], TIGIT [17], 4-1BB, and OX40 [18,19].

3.2. Antibody-Drug Conjugates (ADCs)

ADC is a targeted biopharmaceutical composed of monoclonal antibodies, linkers, and drugs, which uses monoclonal antibodies as carriers to transport small molecule drugs to tumor cells for action. According to the most recent research findings, Trophoblast cell-surface antigen 2 (Trop-2) is a viable target for anti-tumor therapy due to its high level of expression on the surface of feeding layer cells. Trop-2 is a transmembrane calcium signal transduction factor which is represented in humans by the *TACSTD2* gene. It is commonly overexpressed in several forms of malignant epithelial cancers, including breast cancer [20]. Trop-2 actively interacts with multiple critical molecular signaling pathways that have historically been linked to cancer formation and progression, hence contributing to tumor progression.

Sacituzumab-govitecan-hziy (SG) refers to an anti-Trop-2 antibody-drug combination that has showed significant therapeutic results in patients with mTNBC when compared to chemotherapy. PFS and OS were both superior to monotherapy [21]. Patients with moderate or high Trop-2 expression showed the best efficacy. In a clinical trial including 468 patients, 235 were given SG and 233 were given chemotherapy (median age 54 years, all previously treated with paclitaxel). The mPFS with SG treatment was 5.6 months, 3.9 months higher than chemotherapy. Three patients in each group died following AEs, but none of the deaths were considered related to SG treatment [21]. AEs associated with SG in the treatment of mTNBC include neutropenia, vomiting, nausea, and dyspnea [22].

3.3. PARP inhibitors

PARP inhibitors are pharmacological inhibitors of poly ADP-ribose polymerase (PARP) that have been shown to have a significant effect in BRCA-related MBC and to enhance PD-L1 on the tumor cells, blocking T cell-mediated tumor killing [23]. Although PARP inhibitors are targeted drugs, combining them with checkpoint inhibitors can restore immune recognition in PARP inhibitor-treated breast cancer patients. Two regularly used monotherapy PARP inhibitors are olaparib and talazoparib. Table 1 displays the clinical data for both of them.

Olaparib, with the chemical formula $C_{24}H_{23}FN_4O_3$ and the chemical structure depicted in FIG.2, is an enzyme involved in DNA repair that is used to block PARP. In the PETREMACII experiment, patients with primary TNBC more than 2 cm were given olapani alone for 10 weeks before chemotherapy. 18 of 32 patients (56.3%) exhibited an objective response to olapani. Aside from hereditary HR mutations, olapani had a high clinical response rate, fewer adverse events, and a low likelihood of fatigue [24]. Another combination of vastatin with olapani and paclitaxel therapy has been described above.

Table 1. The clinical data of Olaparib and Talazoparib.

Drug	clinical research	therapeutic regimen	mPFS (mth)	ORR (%)
Olaparib	OlympiAD (49.6% TNBC)	Olaparib vs chemo-therapy	7 vs 4.2	59.90% vs 28.80%
Talazoparib	EMBRACA (46%TNBC)	talazoparib vs che-motherapy	8.6vs5.6	62.60%vs 27.20%

Talazoparib, with the chemical formula $C_{19}H_{14}F_2N_6O$ and the chemical structure shown in FIG.2, is an oral PARP inhibitor for treating advanced breast cancer with germline *BRCA* mutations. In a TNBC-containing test using aviote monoclonal antibodies and tarazopanil, the ORR was 18.2% and the median duration of remission (MDOR) was 11.1 (3.4-20.4) months; The ORR in the *BRCA* mutation and DNA damage repair (DDR) positive breast cancer cohort was 34.8%, and eight of nineteen patients got relief with DDR positive tumors, including six patients with *BRCA1/2* mutant tumors, with an MDOR of 15.7 months. When the data was shut off, the response of three patients with *BRCA* mutant malignancies was nevertheless underway [24]. Anemia, thrombocytopenia, and neutropenia are the main AEs. The combination of Avelumab and Talazoparib has a good promise for treating TNBC, according to this experiment [25].

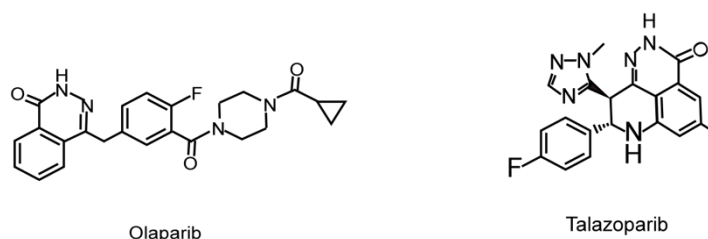


Figure 2. The chemical structure of Olaparib and Talazoparib.

4. Other novel immunotherapies

Other novel immunotherapeutic methods, such as cancer-testis antigens (CTA), chimeric antigen receptor-modified T cells (CAR-T), RNA vaccines, as well as novel antigen vaccines, are still under investigation and some clinical trials have revealed promising therapeutic prospects in.

4.1. Targeting cancer-testis antigens for immunotherapy

CTA is a tumor-associated antigen that has attracted widespread attention as a potential clinical biomarker in targeted immunotherapy due to its cancer-restricted expression and strong immunogenicity. Immunohistochemistry was used to evaluate the expression of NY-ESO-1, MAGEA1, MAGE-A4, KK-LC-1, and PRAME in 51 TNBC patients. KK-LC-1, MAGE-A4, and NY-ESO-1 expression was substantially greater in TNBC than in non-TNBC; 76.47% of TNBC had at least any one of the five CTAs. Patients who have MAGE-A4 or PRAME expression demonstrated substantially longer disease-free survival [26].

4.2. CAR-T therapy

CAR-T therapy refers to a more precise and efficient immunotherapy. The key barriers to treatment progress include molecular heterogeneity, low immunogenicity, and a lack of effective medicines, which the CAR-T therapy method effectively overcomes. This method combines resonant stimulation with T cell effector function. Although advances in the therapy of resistant hematologic malignancies have been made, there are still significant hurdles in the application of CAR-T therapy in solid tumors. The following are the primary barriers to CAR-T therapy in the treatment of solid tumors: 1. Only a subset of tumor cells contacted by CAR-T express the CAR-T-redirected target antigen; The immune-suppressive features of the tumor microenvironment limit CAR-T antitumor efficacy; 3. The

extracellular matrix is an extremely crucial physical barriers linking tumor cells and CAR-T in solid tumor CAR-T treatment [27]. Many antigens have been discovered as possible targets for CAR-T cell treatment in TNBC through preclinical models and early clinical trials.

4.3. RNA vaccines

RNA vaccines are synthetic vaccines that are relatively safer compared to traditional vaccines. Their role is to use RNA to educate cells to generate antigens. Researchers created nanoparticles (NPs) to deliver mRNA vaccines expressing the tumor antigen MUC1 to dendritic cells in the lymph nodes in order to activate and increase tumour-specific T lymphocytes in an RNA vaccination investigation. CTLA-4 monoclonal antibodies are being combined associated with mRNA vaccines to increase anti-tumor efficacy. When in comparison with either vaccinations or monoclonal antibodies alone, combining vaccines with anti-CTLA-4 monoclonal antibodies can remarkably boost anti-tumor immune responses [28]. This shows that NP-based mRNA vaccines associated with CTLA-4 inhibitors used to treat TNBC could be a promising combination immunotherapy.

4.4. Multi-epitope peptide vaccine.

In cancer immunotherapy, multi-epitope peptide vaccines are receiving widespread attention. Currently, multiple multi-epitope peptide vaccines targeting TNBC are being designed, such as those based on Semaphorin 4A (Sema4A), myeloid zinc finger protein 1 (MZF1), and SOX9. Sema4A is a signaling protein recently identified as a major regulatory factor in TNBC progression; MZF1 is a transcription factor and a carcinogenic inducer of TNBC metastasis; SOX9 is also considered a key regulatory factor in TNBC metastasis. Using appropriate adjuvants and epitope combinations, these research teams identified and evaluated the potential of antigen peptide-induced immune responses against Sema4A, MZF1, and SOX9, and conducted immune simulation studies and computer cloning on the constructed vaccines, indicating that in the target organisms, the designed chimeric vaccines can elicit robust humoral and cellular immunological responses [29-31]. Based on this evidence, these types of vaccines may become a promising therapy for TNBC in the future.

5. Combination therapy

When compared to monotherapy, combination therapy has a greater effect in the therapy of TNBC. Current first-line treatment options typically include making use of immune checkpoint inhibitors in conjunction with chemotherapy, as well as the combination therapy of immune checkpoint inhibitors and PARP inhibitors, as discussed in the preceding section. There are, of course, several different combination regimens.

5.1. Combination Immunotherapy Based on Ferroptosis

Ferroptosis is a type of iron-dependent controlled death of cells characterized by the generation of lipid peroxides that differs genetically and biochemically from other kinds of regulated cell death such as apoptosis. The study team proposed and experimentally verified the combination therapy of GPX4 inhibitor, which inhibits glutathione peroxidase 4 (GPX4), and immune checkpoint inhibition for LAR tumors based on ferroptosis research in the context of breast cancer. A ferroptosis map was created by integrating multi-omics data from a large cohort of TNBC samples (n=465), indicating diverse phenotypes in iron death-related metabolites and pathways in TNBC. It has been demonstrated that inhibiting GPX4 not only promotes tumor iron death but also boosts anti-tumor immunity. Combination therapy with anti-PD1 antibodies and GPX4 inhibitors outperformed monotherapy [32].

5.2. Combination immunotherapy of angiogenesis inhibitors, PD-1 immune checkpoint inhibitors, and chemotherapy

The FUTURE-C-Plus phase II trial was designed to assess the possibility of combining famitinib (an angiogenesis inhibitor), camrelizumab (a PD-1 monoclonal antibody), and chemotherapy in patients who have advanced immunomodulatory TNBC. The objective response rate was 81.3%, and the mPFS

was 13.6 months, with no treatment-related fatalities recorded [33]. This study demonstrates the triple combination regimen's effectiveness, safety, and therapeutic potential in immunomodulatory TNBC.

6. Conclusion

This article mostly provides a synopsis of the medicines and treatments available for TNBC immunotherapy. Although TNBC is the toughest type of breast cancer to fight against, and developing an optimum treatment plan for TNBC patients is a substantial unmet need, it is indisputable that immunotherapy has made significant advances in tumor treatment, providing promise for transforming outcomes for some patients. Significant progress has been achieved in TNBC checkpoint inhibitors and therapeutic medicines in recent years, resulting to the identification of various novel immunotherapies. This investigation and advancement of immunotherapy options holds promise for the future treatment of TNBC. Immunotherapy is still in its infancy, and it faces a number of hurdles that must be overcome and investigated further. Basic and translational research have the potential to create new opportunities for the treatment of TNBC and other cancers that now lack viable pharmacological treatments.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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Nutritional supply: The potential simplest vaccine and therapy as tuberculosis control intervention in China

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Abstract. Tuberculosis (TB) is a highly contagious disease that previously held the distinction of being the leading cause of mortality prior to the COVID-19 pandemic. It imposes a significant burden on a global scale. This disease might potentially impact individuals' regular nutritional status and impose financial strain on patient's family. This article discusses the current state of TB disease burden in China and India, which serve as representative examples of nations with a high burden of this disease. Next, a discussion was held regarding the implementation of ordinary control interventions in China. Considering nutritional support has gained increasing recognition as a novel intervention in the management and control aspect. The potential feasibility of implementing nutritional support as a novel intervention for TB control in China is discussed in this article, drawing upon the success of a trial conducted in India. However, additional research is required prior to the practical implementation.

Keywords: Tuberculosis, nutritional support, intervention.

1. Introduction

Tuberculosis (TB) is a highly transmissible disease which is induced by the bacillus *Mycobacterium tuberculosis* that significantly contributes to the global burden of poor health, especially in low-income or in middle-income countries. Before the onset of COVID-19 (coronavirus) pandemic, TB occupied the position of being the leading cause of mortality from single infectious agent, surpassing even the long-term prevalence of HIV/AIDS worldwide [1]. Individuals with weakened immune system are more susceptible to tuberculosis infection compared to individuals with intact immune system [2].

Due to the accessibility of potent anti-tuberculous medications, it becomes feasible to implement controlling interventions in regions with a significant burden of TB disease. The conventional intervention mode mainly focused on the medical treatment for those with active tuberculosis. However, it is important to note that drug treatment alone does not provide a comprehensive solution to the prevention of new TB infections. Furthermore, the potential consequence following an improperly or incompletely utilization of drug treatment is the development and proliferation of drug-resistant variants, which could potentially give rise to an additional emerging issue in disease control [3]. According to the analysis, current available intervention would not effectively expedite the reduction in TB cases to the desired extent. Consequently, there is a need of developing novel and enhanced intervention in order to expedite the decline in disease incidence and alleviate the global burden of this illness. Relying upon the successful study conducted in India, it is obvious that nutritional support has the potential to serve as a viable strategy in the control of TB on a broader scale.

This paper aims to demonstrate the effectiveness of nutritional support in TB controlling procedure in China following India's successful experience as a model.

2. Two phases in TB disease progression

There are two distinct conditions associated with TB: latent TB infection (LTBI) and TB disease. LTBI refers to a state in which the bacteria can persist within the body without causing any symptoms. In most case of TB infection, the immune system of the individual is able to stop the bacteria's proliferation. As a result, individuals are not capable of transmitting their infection to others. The subsequent stage of TB disease occurs when the bacteria become active, get rid of the control from immune system and start the multiplication within an infected individual. In this period, individuals show syndromes and potentially serve as carriers for spreading the replicated bacteria. Not every individual progresses from LTBI to the TB disease, and the duration of this evolution varies significantly among different individuals. The use of flexible and adaptable treatment strategies could prevent the progression from LTBI to TB disease [4].

3. Global burden of tuberculosis

The current estimation of global prevalence of LTBI is approximately as 25% of the global population. Approximately 10% of these persons have risk of developing TB over their lifetime [5]. In 2020, the prevalence of LTBI on a global scale was estimated to 23.0%, amounting to approximately 1.7 billion infected individuals. Assuming the current LTBI status is maintained, by the years 2030 and 2050, it is projected that the group of LTBI individuals will give rise to 16.3 and 8.3 active TB patients per 100,000 population respectively [6]. The objective for TB elimination is to achieve a annual reduction less than one case per million population by 2050. Achieving that goal necessitates a decrease in incidence by a factor of 1,000 during a timeframe about 35 years [7]. Since the majority of newly reported TB cases arise from the reactivation of LTBI rather than recent transmission, there is a recognized need to enhance treatment approaches for the former. This is considered a vital element in the efforts to control or eliminate TB in areas with a high incidence of the disease [5]. In 2019, it was projected that around 10.0 million individuals worldwide contracted TB disease. Adults comprised 88% of the total number of deaths. Most individual developed to TB disease were in South-East Asia (44% of total infection cases, WHO regions).

There exist also wide variations in the prevalence of LTBI across regions. Approximately 66% of the global new infection cases was attributed to eight nations, where India accounting for 26% and China accounting for 8.4% [6]. Despite the downward trend in TB incidence in these two countries, as indicated by epidemiological statistical data, it remains a significant issue [8]. Both of them are still listed as high burden of TB and other related disease countries for 2022 in WHO TB global report [1].

4. “Vicious circle” between TB and malnutrition, and drug-resistance problem in TB prevalence

TB is associated with numerous negative impacts on health status, including the decrease of appetite, nutrient absorption and metabolism capacity, resulting in wasting and a decline in the general state of nutrition [9]. A “vicious circle” could be observed between TB and malnutrition. Existing of TB would contribute to malnutritional status by causing deficits in both protein-energy and micronutrient way. Consequently, this malnutrition further enhances the susceptibility of the host to TB infection. Research has indicated that lack of nourishment could affect both innate then adaptive immune responses that is required in controlling TB infection and may also impact response to live vaccinations for example BCG. As an example, malnourished mice received BCG vaccination had a higher level of mycobacterial dissemination to the lymph nodes and thymus compared to well-fed ones. This finding has raised concerns over the potential negative impacts of vaccination in malnutritional status [10]. TB patients with malnutrition experience a prolonged recovery period and elevated mortality rates in comparison to patients who are appropriately nourished [11]. In TB pandemic countries, undernutrition act as a virtual driver in increased TB incidence or prevalence, as well as in the poor outcomes of therapy [110].

The potential severity of the prevailing malnutrition issue may surpass public's initial perceptions. Globally, the number of individuals suffering from chronic undernourishment is estimated to be around 800 million. 98% of these individuals reside in low-income and middle-income countries where TB also in endemic [10]. For example, in India, TB and undernutrition coexist as syndemics [12].

The use of drug treatment alone as a conventional control method itself is insufficient in preventing the reactivation of TB within the extensive population of LTBI individuals. Furthermore, improperly or incompletely usage of this approach may potentially contribute to the issue of drug resistance [3]. Recently, an increasing focus on nutritional support has been presented within the field of TB disease control. A cluster-randomized controlled trial was conducted in four districts of Jharkhand in India to investigate the effectiveness of a field-based, open-label intervention. The experiment involved household contacts of 2800 patients who had microbiologically confirmed TB infection. These previous studies have demonstrated that implementing a dietary intervention in only 6 months resulted in 39%-48% over a span of two years as a significant decrease of TB incidence within the household [12]. In conclusion this successful trail illustrates that the use of nutritional supplementation could potentially be a beneficial strategy in the management and control of tuberculosis.

5. A succeeded study application in India [12]

Previous trials have demonstrated that nutritional support as a “biosocial intervention” can effectively facilitate the reduction of TB incidence in TB and undernutrition syndemic countries. During the period from 1918 to 1943, partial area in UK faced the challenge of TB without the availability of chemotherapy or the BCG vaccine. The enhancement of living conditions, which subsequently led to improved nutrition, resulted in a significant reduction of TB incidence by a factor of six.

A clinical trial was conducted in India from 2019 to 2022, applying a total of 10,345 home contacts as experimental samples. 3543 (34%) of the entire experimental population experienced the condition of undernutrition. The total number of cases was randomly and equally allocated to either the control group or the intervention group. The distribution process was consistent to an average household size of one index case, along with four contacts in each group. In both 2 groups, TB patients were provided with a monthly food supplement containing 1200 kcal and 52 grams of protein per day, as well as daily pills delivering the required dietary amount of micronutrients. The intervention group's family contacts were provided with an extra food supply containing 750 kcal energie supply with 23 grams of protein per day and per person, as well as a recommended dietary allowance of micronutrient pills. An extensive list of participants' basic information, categorized by groups, as well as their average values in aggregate, is provided in Table 1.

Table 1. Basic information of participants of household contact trial in two groups. (Not original, data from [12])

Groups and respective participants number	Intervention group (n = 5621)	Control group (n = 4724)	Total (n = 10 345)
Cases of TB incidence	96 (1.7%)	122 (2.6%)	218 (2.1%)
Contacts / index case (1400 index patients in each study group)	4.0	3.4	3.7
Presence of BCG scar Total	3426 (61.0%)	2557 (54.1%)	5983 (57.8%)
Asset cost Cost of 23 household assets (American dollar on actual exchange rate in 2023)	16 500 Indian Rupees Approximately 198 \$	15 000 Indian Rupees Approximately 180 \$	

A decrease observed in the tracked total population each group as a result of the COVID-19 outbreak. The primary outcome assessment was completed by 5328 (94.8%) cases in the intervention group and 4283 (90.7%) cases in the control group, as a result of practical limitations. In this study, investigators have observed that the cumulative incidence of tuberculosis increases in each of the two groups as time progresses. The detailed results of accumulated incidence in two groups respectively could be found in figure 1 and table 2.

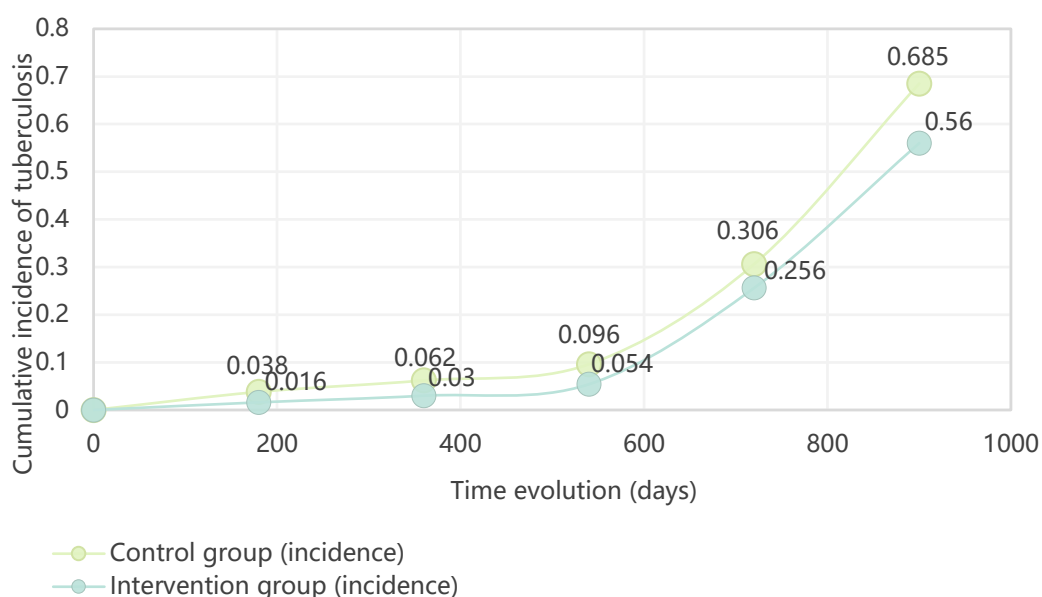


Figure 1. The cumulative incidence of TB disease within the trial group over the duration of the follow-up period, visualized by a scatter plot. (Not original, data from [13])

Table 2. Basic information of participants of household contact trial in two groups. (Not original, data from [12])

Group	Number (decrease progressively over time)					
Control group (total number)	4631	4619	4592	4562	4539	4526
Intervention group (total number)	5542	5538	5512	5495	5476	5463
Control group (incidence)	0 (0%)	174 (3.8%)	283 (6.2%)	437 (9.6%)	1387 (30.6%)	3100 (68.5%)
Intervention group (incidence)	0 (0%)	88 (1.6%)	167 (3.0%)	296 (5.4%)	1400 (25.6%)	3058 (56.0%)

In beginning data, it is evident that among the undernutrition population, the initial occurrence rate of TB surpasses that of the general population (where nutritional status is determined by individual weight as shown by body mass index, or BMI). The variation between the data obtained is about a twofold difference. This randomized controlled trial demonstrates the effectiveness of nutritional support in significantly reducing 39% of the TB incidence rate across every form of the disease. Additionally, the intervention group exhibited a notable 48% decrease in the incidence rate of microbiologically confirmed pulmonary tuberculosis as the principal infection mode, compared to the reduction in control group. In summary, the nutritional support has been demonstrated to be a cost-effective, efficient, and accessible method in controlling TB, as evidenced by empirical research conducted in India. The final comprehensive outcome data is accessed in Table 3.

Table 3. The conclusive data provided within two trial groups. (Not original, data from [12])

	Incidence per 100 person per year (caculated in %)	
Group	Control group	Intervention group
Nutrition status at baseline Underweight (BMI < 18.5 kg/m ²)	1.94 (1.48–2.54)	0.75 (0.51–1.12)
Nutrition status at baseline Normal or above (BMI ≥ 18.5 kg/m ²)	0.93 (0.66–1.31)	0.33 (0.17–0.65)
Overall	1.27 (1.00–1.61)	0.78 (0.64–0.96)

6. Current situation of tuberculosis and related controlling intervention in China

6.1. The actual burden of TB disease in China

As previously mentioned, although there has an obvious decrease in the tendency during the past few years, China continues to face a significant burden of TB disease. China accounts for 8.4% of the global new incidence cases, indicating that it ranks third among countries with the largest burden of tuberculosis worldwide [1]. Another issue that has arisen since the onset of the COVID-19 outbreak in 2019 is the diversion of primal healthcare resources and accompanying infrastructure away from other problematic diseases, such as TB. Based on predictions provided up by relevant researchers, it is anticipated that the COVID-19 pandemic will result in a 20% rise in tuberculosis mortality worldwide within the upcoming five-year period [11].

6.2. Mainstream disease control interventions in China

The dominant strategy for TB control in China continues to be the utilization of traditional methods. This involves providing vaccinations prior to potential exposure, followed by medical treatment after affirmative infection. In China, children under the age of one are eligible to receive free TB vaccine (BCG), in order to reduce the risk of future infection. Furthermore, individuals diagnosed with TB receive a fundamental set of free testing and medications. The expenses associated with additional services are borne by publicly-funded medical insurance programs. According to a study involving a total of 3,286 respondents diagnosed TB, the median costs associated with TB care per patient were found to be 1041.3 USD, 12.7 USD, and 1185.5 USD for direct, indirect, and total costs, respectively. The proportion of direct expenditures in relation to overall costs was found to be 88.0%, whereas direct medical costs constituted 69% of the total costs. One notable inference that can be drawn from the context of Chinese public health practice is the substantial financial burden faced by both the government and individual TB sufferers. The existing tuberculosis (TB) care strategies and packages are inadequate in effectively addressing this matter [13].

Another global issue that significantly impacts TB therapy in China is drug resistance. China is currently dealing with a significant burden of drug-resistant TB and multidrug-resistant tuberculosis (MDR-TB), which has resulted in it having the second highest caseload worldwide. MDR-TB exhibits a disproportionate impact on impoverished rural communities, with the highest prevalence observed in less developed locations. This resistance can be attributed mostly to the interruption and/or inappropriate implementation of TB treatment protocols. As previously indicated, a significant financial burden is experienced by the majority of households affected by the condition, primarily due to the cumulative expenses associated with treatment and other related expenditures. As a result of this factor, the application of TB treatment was restricted, leading to inadequate implementation or premature discontinuation [14].

7. Deduction: Why the application of nutritional support will be feasible in China?

In the aforementioned trial conducted in India, it has been demonstrated that nutritional support is a viable, efficient, and cost-effective intervention in the TB control or treatment. As an alternative of

medical supplies, the cost of food supply would be considerably less. The potential benefit includes not only the economic advantage, but also a potent countermeasure towards medication resistance.

The management of TB in India is confronted with three major challenges. Firstly, disordered private healthcare sector, which is often characterized by a lack of organization, but serves as the initial point of care for a majority of patients. Consequently, patients may not always have access to adaptable and appropriate diagnosis or therapy following TB exposure. The absence of coordination and information sharing between departments restricts effective collaboration and communication, resulting in limited dissemination of information to public health authorities. Due to a lack of information pertaining to the present state of the disease, the government encounters challenges in formulating pertinent control policy for TB control. Lastly, the current economic load poses complications for the government in allocating funds towards nutritional support initiatives [15].

On the contrary, the Chinese government's demonstrated execution ability in combating the initial outbreak of COVID-19 in 2019, coupled with its remarkable efficiency in epidemic control, indicate that the medical system in China possesses the capacity and potential to achieve comparable results in managing other diseases.

When comparing the two countries, it can be observed that China possesses a marginally higher Gross National Income (GNI) in contrast to India [16,17]. As previously said, it is important to note that the Chinese government is currently assuming partial financial responsibility for the diagnosis and treatment of tuberculosis patients. This implies that the government possesses the capability to assume the estimated cost of nutritional support as an intervention, which is far lower than the former amount.

In conclusion, it is recommended that the Chinese government explore the practical application of nutritional support as a simple, practicable, successful, and low-cost innovative approach to control tuberculosis.

8. Conclusion

Tuberculosis (TB) is an important topic in infectious disease context, having imposed a substantial burden on a global scale for an extended period of time. Present measures for controlling TB have been found to be unsuitable for the current circumstances and are unable to meet the public's demand for combating the prevalence of TB. The implementation of nutritional support offers a novel approach that can provide patients with the essential nutritional requirements. This intervention has the potential to contribute to disease prevention, delay disease development, and give therapeutic advantages in the context of tuberculosis control. Based on the findings of a practical study conducted in India, it has been suggested that nutritional support could potentially serve as a novel and cost-effective intervention in China, potentially leading to superior outcomes compared to those observed in India. However, the practicality and availability of implementing this proposed intervention are still being assessed through a series of studies in order to get formal approval.

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Comparison of TB in India and Japan

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Abstract. Tuberculosis bacterium (TB) is still a major world public problem, especially in Southeast Asia. Data in 2021 from WHO showed that 10,600,000 population infected with TB and more than 10% among them died. Tuberculosis, as a bacterial disease, is capable of infecting different organs of human body, especially lung, which cause many serious symptoms and even death. It can spread through the air or small liquid drops that produced by breath, speaking and so on. This review firstly describes the characteristic of TB infection in India and Japan, compares the past tuberculosis situation between the two countries, and summarizes some shortcomings of TB control in India including lack of nationwide surveys, lower vaccination coverage, and low-quality welfare system to the health of citizens. The purpose is to provide some possible solutions for India to reduce TB from learn the experience of prevention and control in Japan, such as increasing TB education and providing nutritional supplements to undernourished people.

Keywords: Tuberculosis, India, Japan, comparison, solutions.

1. Introduction

Data in 2021 from WHO showed that 10,600,000 population infected with tuberculosis bacterium (TB) and more than 10% among them died, especially serious in Southeast Asia [1]. And some population contract TB with higher risk, such as HIV patients, medical workers, elders, and people in centralized management including prison, nursing house and so on) [1].

Tuberculosis, as a bacterial disease, is capable of infecting different organs of human body, especially lung [2], which cause many serious symptoms and even death. It can spread through the air or small liquid drops that produced by breath, speaking and so on [2]. Most people infected with TB, belong to latent infection without symptoms although the bacterium exist in bodies of infected individuals, whom possess positive skin/blood test reaction, but can't infect others. The incubation period is about 3 to 9 weeks from primary lesion to obvious tuberculin reaction [3]. If they do not receive appropriate treatments, some of them will develop tuberculosis and infect others, especially in the condition of vulnerable immune system [3]. According to a survey in 2023, the symptoms of TB including cough with or without blood and mucus, chest pain with breathing, night sweats, and so on [3].

2. Tuberculosis in India

The characteristics of high TB burden countries or regions is high incidence more than 100/100,000 according to WHO handbook [1]. India is known to be one of the highest TB burden countries, which incidence is higher than that. What's more, India belongs to lower middle-income country according to data from the World Bank. In 2019, India had 2,640,000 cases, and 436,000 of them died from TB, accounting for one quarter in the world [4], which still has a long way for them to meet this goal of reduction 80% incidence at 2025 although the incidence has been decreasing (Figure 1) [5]. The incidence has reached the lowest level since 2015, but it still above the threshold of 100/100,000 population incidence. In addition, the incidence of tuberculosis increased again in 2021, higher than the past few decades. The main features of TB infection in India are that the incidence of TB increases with ages both in males and females, and males is higher than females in the most age groups, especially in people over 80s [5]. It's to be noticed that the relative incidence declines between the 65 and 69, although the incidence of this group is still higher than the under 50s groups [5]. The survey in 2021, involved 69054 eligible people to take chest X-ray to check if they have symptoms of TB infection, supplied more information of TB in India. The researchers divided the volunteers into three age groups including group of 15 to 34, group of 35 to 54, and group of over 55. The result showed that people over 55 have the highest rate of TB (969 people, 7.1%) in comparison of the groups of 15 to 34 age (116 people, 0.4%) and 35-54 age (476 people, 2.1%) [6], demonstrating that the elder people are at higher risk of tuberculosis.

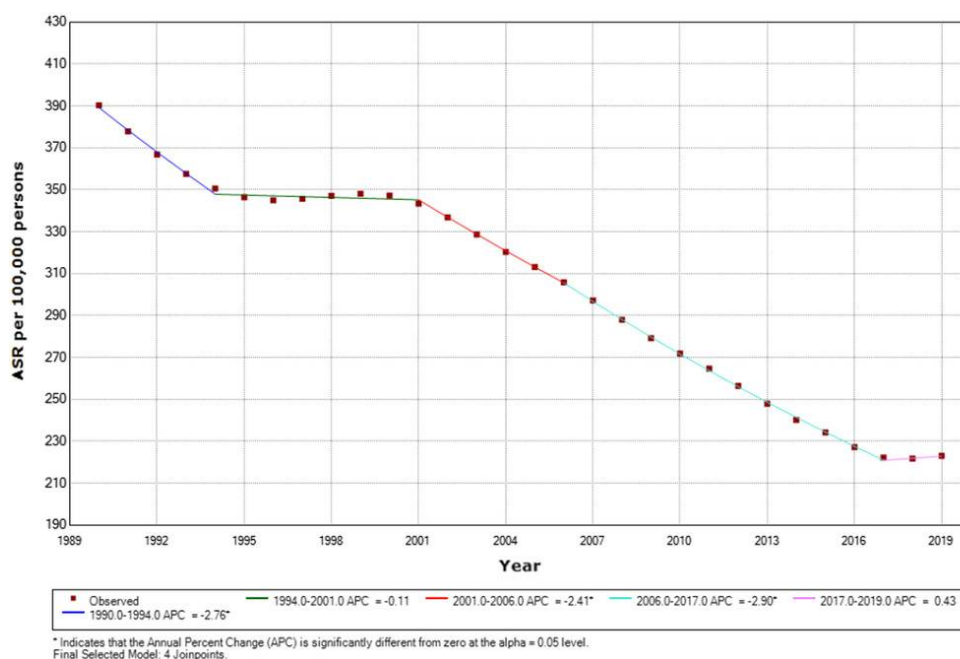


Figure 1. Incidence trends in both sexes of India between 1990 and 2019 [5].

Another phenomenon of TB in India is that the prevalence varies greatly in different places. For example, the prevalence was the highest in the state of Arunachal Pradesh (840/100,000 population), whereas the prevalence was only 50/100,000 population in Daman and Diu [7]. In addition, a study of the relationship between multidimensional poverty and tuberculosis in India found that the prevalence of tuberculosis in population of multidimensional poverty was 4.80‰ compared to 2.50‰ among the non-multidimensional poor [7]. A great amount of people are suffering from malnourished in India. More than 50% of TB cases are influenced by under nutrition [8], demonstrating the association between TB and poverty.

3. Tuberculosis in Japan

The epidemic of TB is worse in Asia than in Europe. For example, Japan, managing a high-level medical service, still have an incidence of 10-100/100,000 population with mid-burden. For example, the incidence was 16.7/100,000 people, much higher than western countries with 4-5 fold, although the government had made many efforts to handle with tuberculosis [9]. The study of Lu et al showed that newly reported infections decline slowly from 364 to 296 per 1,000,000 population during 2007 to 2018 [9], with a little peak between 2007 and 2013 (Figure 2), then sharply reduction, which is quite different with the pattern of India. Similar to India, the males have higher incidence than females in TB and the incidence rate increases with the ages in Japan . More than 60% of TB patients are people over 65 [9], especially in men aged > 85. In addition, 68.4% of TB patients are unemployed [9].

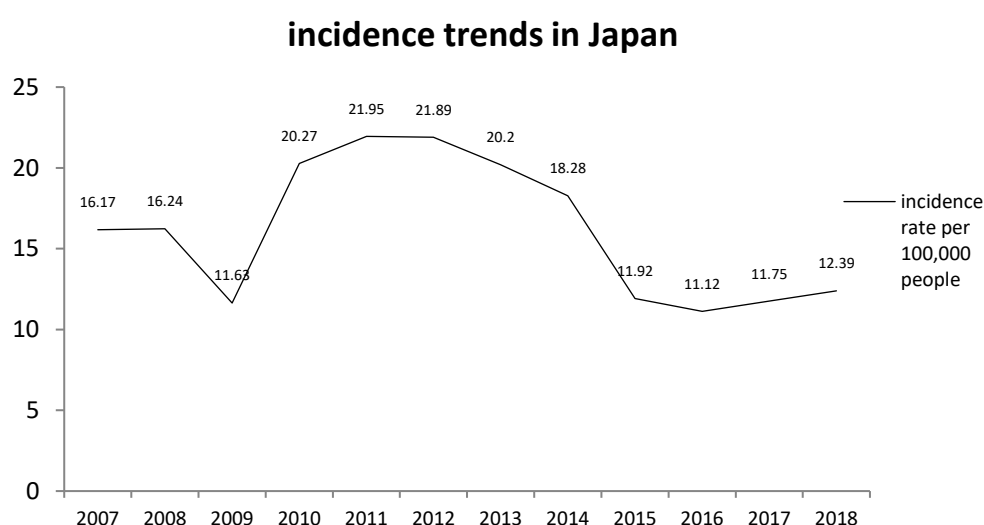


Figure 2. Annual incidence rate in Japan from 2007 to 2018 [10].

A study in 2021 showed another characteristic of TB infection in Japan is that the number of TB cases in different parts of Japan varies greatly, which is similar to India. For example, Tokyo had 120 outbreaks, then Osaka (62), Kanagawa (37), Aichi (35) and Fukuoka (31), whereas the relative low ratio located in Tottori, Tokushima and Yamaguchi (all 2), and Fukui and Yamanashi (both 0) [10]. In addition, the number in workplace is relatively high, which is highest significantly in nursing center and psychiatric hospitals [10]. Meanwhile, there also some relates between season and TB outbreaks in Japan. The TB cases were more often broke out in winter [10] since the windows are closed to keep warm, supporting TB transmission indoors. In addition, it is confused that the number of TB outbreaks increased in last two decades. A possible explanation is that TB cases stood out and easily to be detected with the decrement of TB incidence.

4. The comparison in TB between two countries

As the information mentioned above, there are many differences about TB between India and Japan. Firstly, Japan is a medium TB burden country but India is a high TB burden country. The incidence of TB in India in the past few decades was much higher than Japan. The TB incidence is (223-390)/100,000 from 1990 to 2019 in India [5], whereas the incidence of TB in Japan was no more than 16.7 per 100,000 people until 2012 [9]. Although the Indian government put so many efforts on TB prevention in the past few decades and achieved some goal, they still remain one of the highest TB burden countries in the world. These reasons may explain the above difference. Japan conducts regularly including the elderly population and immigrants, while India lacks the nationwide TB survey

[11]. Another reason is that Japan has better economic background and welfare-system than India, resulting in Japan can quarantine and heal TB patients more efficient, eventually help Japan reduce the spread of epidemic. In compassion with Japan, India's poor public-private health partnership adds to the burden of TB in India [5].

Secondly, the cases in India broke out more in rural areas while the cases in Japan were often found in urban. Of the 605 incidents from 1993 to 2015, 120 were found in Tokyo [10]. In Japan, the workplaces are the most common places for TB outbreak following by health and welfare facilities [10]. These facilities are more common in big cities. This phenomenon happened because these facilities keep the windows closed to keep warm during winter, which enhances the spread of TB. Family infections are present in Japan but is much lower than in the above facilities [10]. In India, more studies concentrated on rural areas. In these areas, people experience more TB infections. For example, a community survey in south India showed that more than half people had not been vaccinated against BCG [6]. Another reason is the lack of awareness of TB in rural areas, which many people do not report TB infections in time because of stigma. At the same time, small clinics in India villages also lack effective diagnosis and isolation [6], leading to the spread of TB in families and communities in Indian rural areas. In addition, the 14 percent of Indian's population suffers from malnutrition according to the World Bank report in 2018 [8]. And more than 50% of TB patients are malnutrition [8], especially in rural areas.

Thirdly, the incidence of TB in India has declined continuously since 1990s [5], but the incidence of TB rose up during 2007 and 2013 and fell down sharply in Japan [9]. The following reasons may lead to this phenomenon. The TB situation in India is much more serious than Japan, and it is easier for the Indian government to reduce the same number of TB cases as Japan, leading to more obvious effects than Japan. In comparison to India, Japan's data as a whole is much more complete. What's more, the attention to TB in Japan has slackened in the past few years [9]. Although TB in Japan has fluctuated somewhat, it is still far better than India's overall.

But there are also some similarities between two countries in terms of TB. Firstly, those at high risk of TB infection are older people in both societies. Moreover, TB infection is both more severe in male than in female [5, 9]. Secondly, there are some similarities in the populations where the outbreaks occur although there are some differences in locations of TB outbreaks in two countries. In India, multidimensional poverty populations have higher risk than non-poor populations [7]. The prevalence of TB among the multidimensional poor was twice compared to non-poor population [7]. The multidimensional poverty includes under nutrition, poor education and uptight standard of living, such as no access to improved sanitation facilities, to clean water, and to use "dirty" cooking fuel and so on. Similar to India, 68.4% of TB patients were unemployed with a relative low living conditions during 2007 to 2018 [9].

5. Possible solutions to India

In Japan, nearly all the young people have received BCG vaccine. Although BCG is not a very effective vaccine and does not protect well against primary infection in adult, it still protects against disseminated forms of TB cases in kids [12]. A study in India also showed that people vaccinated with BCG had a much lower rate than those not vaccinated [6]. The data from more than 50 countries and over 95% coverage showed that BCG vaccine approved for application in clinic of the worldwide [12]. The BCG vaccine, which has been used for more than 100 years, is cheaper than developing new vaccines and it still be used in countries of high TB burden such as India. Japan is a medium TB burden country, which BCG coverage is close to 100% [9], suggesting India should do more to promote vaccination among at-risk groups.

In Japan, certain population is regularly tested, including people in mental hospitals, nursing homes, prisons and so on [9]. And the Ministry of Health, Labour and Welfare adopted the WHO's new strategy in 2014, considering widespread screening for latent TB infections, especially among the elderly and some migrants, and strengthening TB control in large cities based on geographical differences [9]. In India, regular TB testing still have many shortcomings and areas have not been

adequately investigated [11]. The TB situation in India varies greatly and more population-based surveys across the country are needed to help the government plan and control more efficiently in India.

Another area that cannot be ignored is civic education. In recent years, Japan strengthened the publicity and education of TB prevention since the concern of Japanese citizens about TB has declined. Moreover, the higher education level of citizens in Japan is conducive to the promotion of epidemic prevention knowledge. India has a large number of people without formal education, so they have a poor understanding of TB and how to treat it [7]. Many people do not avoid the high risk of infection in India, which promotes the spread of disease. It is necessary for India to advocate widely the knowledge of prevention and control TB to reduce the incidence and prevalence of tuberculosis.

Japan has a better welfare system and protecting public's health is a very key part of welfare. Complications such as malnutrition and parasitic infection are common in TB-endemic countries such as India [8]. Providing nutritional supplements to the public is an option worth considering to India government. Malnutrition leads to a deficiency of the innate immune system, which is made worse by common parasitic infections in India. Follow-up of the blood samples in a study showed that the malnourished participants had increased immune response levels after continuous nutritional supplementation [8]. This makes a lot of sense in India where medical resources are scarce. Improving the immunity of the population can not only reduce the new TB cases, but also improve the success rate of TB control in the affected population.

6. Conclusion

Although India has made some progress in TB control in the past few decades, it is still far from India's target of 80% reducing of TB incidence by 2025. Japan has some similarities to India in the characteristics of TB infected population and has much better TB control. India can learn from Japan's experience to narrow down the TB epidemic. India should spend more energy on enhancement of vaccines, spreading TB knowledge, increasing TB research and improving the health of its people in the future. But in this paper, some details of TB in two countries are still different. For example, there is some lag in the TB research data of two countries, more recent information is unclear. Japan's experience for India can only be used as a reference, which needs to develop measures that are more tailored to the actual situation.

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Role of digital epidemiological technologies in controlling COVID-19: Cases review

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Abstract. The pandemic of COVID-19 had not only been testing the existing health care system globally, but also highlighting the need for more innovative and adaptive methods in dealing with new health threats. Digital technology become one of the most effective and efficient strategies in terms of controlling and monitoring the outbreak of this disease. These digital strategies will serve a significant role in providing valuable insights in disease and virus control for any upcoming event. This paper aims to discuss and evaluate the roles of digital technology methods in addressing COVID-19 pandemic, while focusing primarily on two case studies: COVIDSafe App from Australia and the EU's Digital COVID Certificate. While confronting the challenge raised by COVID-19, the world had invented critical digital tools as instruments to monitor, track, and manage the spread of virus. By taking a deeper look at the history, technology, and application of the successful cases, the author hopes to draw important lessons from these experiences, and foreshadowing some broader potentials for their future development and usage. Additionally, the author also analyzes the legal and privacy concerns that may come with the adaption of digital technology in fighting the pandemic, and underscore the implication of technological methods advancement for future health crisis.

Keywords: Digital Epidemiology, COVIDSafe, EU Digital COVID Certificate, Digital Technology.

1. Introduction

In early 2020, an unprecedented crisis emerged from China, spreading rapidly to the rest of the world in a fashion that had yet to be seen in modern health care and public health fields. Later identified as COVID-19, the pandemic had not only been testing the existing health care system globally, but also highlighting the need for more innovative and adaptive methods in dealing with new health threats. Despite the uncertainty, digital technology had found its way in becoming one of the most effective and efficient strategies in terms of controlling and monitoring the outbreak of COVID-19. These digital strategies will serve a significant role in providing valuable insights in disease and virus control for any upcoming event. This paper aims to discuss and evaluate the roles of digital technology methods in addressing COVID-19 pandemic, while focusing primarily on two case studies: COVIDSafe App from Australia and the EU's Digital COVID Certificate. While confronting the challenge raised by COVID-19, the world had invented critical digital tools as instruments to monitor, track, and manage the spread of virus. By taking a deeper look at the history, technology, and application of the successful cases, the author hopes to draw important lessons from these experiences,

and foreshadowing some broader potentials for their future development and usage. Additionally, the author analyzes the legal and privacy concerns that may come with the adaption of digital technology in fighting the pandemic, and underscore the implication of technological methods advancement for future health crisis.

2. Overview of digital epidemiology

Digital epidemiology has been on a rapid rise for the past decade [1], data generated from all sorts of sources have been accumulating, which could be used for the purpose of public health. But firstly, it is necessary to address the question of what is digital epidemiology. “Digital” being the key part of the term digital epidemiology frames the direction of the definition. Dr. Salathe defined digital epidemiology in his article as epidemiological work that utilizes data not originally generated for doing epidemiology [1]. These data sources include but not limited to social media (Instagram, Facebook, and etc.), search engines, cellular data, data captured by sensors, and many more [2]. These data are to enhance the existing epidemiological approaches and to adapt to new public health threats more rapidly.

One of the most well-known early digital epidemiology examples is Google Flu Trend [1]. It aims to analyze flu outbreaks using search queries [3] and demonstrated the potential of such digital methods being successful in helping health care professionals and epidemiologists in disease surveillance. However, there were issues with Google’s approach into digital flu monitoring. Over-estimation and lack of transparency are the main problems that Google Flu Trends were having [1, 3]. But it ultimately underscored the need for further refinement in digital epidemiological methods. As time passed and technological advancement took place in the following years, the employment of more enhanced tracking, data collection, and data analysis ability eventually lead to various application of digital epidemiological strategies against COVID-19.

In the present, the author categorizes some key components of digital epidemiology in this section. As there are mainly three critical parts of every digital epidemiological method: data collection, data analysis, and data integration. Data collection refers to the gathering of data from various sources, these data could be numbers, locations, and etc. Data analysis involves the processing of data collected, such mechanism could employ machine learning, digital data analysis, and many more in order to extract valuable insights. The last step, data integration, calls to the application of the results of data analysis. Devices like smartphones could potentially benefit significantly from the data to carry out applications that helps controlling any health threats (Figure 1).

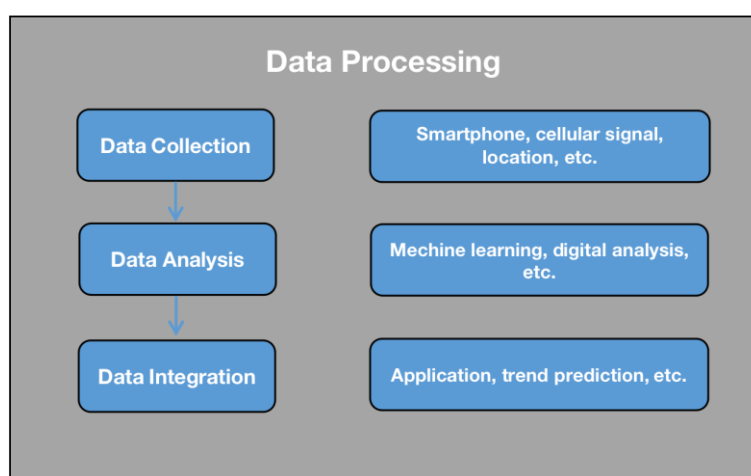


Figure 1. The main data processing of digital epidemiology.

3. Application of digital technologies in epidemiology

3.1. Case one: COVIDSafe

COVIDSafe tracking application for smartphones was developed and launched by the Australian government with the purpose to combat against the spread of COVID-19 virus in Australia. The app mainly has the function of tracing existing infected cases, and notifying potential contacts. Bluetooth and smartphone technology had played an critical role in enabling the successful operation of the concept of real-time tracing app like the COVIDSafe. Individuals were able to be notified if they were in close proximity to a confirmed case. This was done through Bluetooth signaling by each signed-up app user's phone. However, despite its effective functions, there remains some concerns surrounding the privacy issues of COVIDSafe.

The function of COVIDSafe's tracing and tracking mainly rely on Bluetooth technology. Bluetooth data exchange also ensures that each user's private data would not be shared or leaked to unwanted parties. The basic principle of how COVIDSafe works involves the exchange of data between smartphones. When two individuals come into close contact, their smartphones would initiate data exchange, these interaction is encrypted as to protect user's privacy. Information being exchanged will also solely be stored on individual devices' local storage. If an individual has been confirmed positive for COVID-19, they have the option to upload their infection status to a government server. Therefore, individuals could be notified when they have been in close contact with a confirmed case, hence being able to seek further help from health authorities.

The app COVIDSafe is significant in terms of being a crucial tool in containing and controlling the spread of virus in Australia. Its aim was to reduce transmission and prevent potential outbreaks. The application offered a more streamlined way to employ tracing and tracking within communities. Being able to trace close contacts and noticing them in a short time frame is critical for breaking the chain of infection in a epidemiological outbreak. Also, COVIDSafe served as a tool to help authorities to reach a broader population when it comes to tracking of health. It is extremely time-consuming and less effective to manually record and track transmission data, especially in more crowded areas. COVIDSafe had become a successful digital methods to resolve challenging tasks in a rapidly spreading and highly contagious pandemic.

However, although the COVIDSafe application had its success in areas of tracking, it did also face some concerns and limitation about uptake rate and individual user's privacy protection. A tracing application rely heavily on the total amount of users who download and use the app, as the more people have the app, the more data it can collect and analyze. However, the uptake and adoption rate of COVIDSafe app was not as high as anticipated [4]. Data has shown that about one-third of the participants in a survey about COVIDSafe app had not downloaded the application due to multiple reasons, including lack of health concerns, technical difficulties, privacy concerns, and more [5]. Without a large population pool, tracking app like COVIDSafe might fall short of performing as good as predicted. Additionally, there have been concerns about data privacy from individuals who chose not to download the app. This factor covalently attributes to the reason why there is a lower-than-expected uptake rate. A survey carried out by Thomas revealed that among those surveyed, 25% did not download the COVIDSafe application due to privacy concerns (2020) [6].

Overall, COVIDSafe tracking application serves an important role in controlling and monitoring the spread of COVID virus in Australia. It eased the pressure on health authorities in terms of data collection, case tracking, and exposure notification. However, there are lessons to be learned from this experience. Uptake rate among citizens is critical for the performance of a tracking application, governments have to address the issues around people's concerns on privacy, technical difficulties, and data process in order to generate better strategies for the future [6].

3.2. Case two: EU Digital COVID Certificate

In response to the emergence of COVID-19 virus in Europe, the Europe Union (EU) approved and launched a crucial digital strategies named "EU Digital COVID Certificate" in 2021, aiming to

facilitate movement and control the spread of virus within EU countries [7]. This move had been discussed and debated among EU members for a long period of time before passing [7], the application of this digital certificate would be beneficial for free traveling of people within the EU countries, without any more restrictions like quarantine or testing [7]. This method is important in maintaining the freedom traveling, boosting harmed economy, and controlling spread of COVID-19 virus.

The EU Digital COVID Certificate involves three main certificates. First one being a vaccine certificate, which documents the status of vaccination of an individual [7]. The second one is a certificate for recent testing. This certificate includes the detailed data about an individual's recent COVID-19 test, which could be PCR or rapid test [7]. And finally, a certificate for recovery, as one could potentially have recovered from a COVID-19 infection [7]. These component of the EU COVID Certificate enables the possibility of authorities across the EU to conveniently check and monitor one's health status. Additionally, the appearance of QR code generated when completing the certificate offers an efficient way for travelers to easily showcase their status to authorities, hence providing a free and swift experiences while traveling.

The significance of the EU Digital COVID Certificate in guaranteeing free traveling and disease control cannot be overlooked. It raised an effective way to balance the need for traveling and virus control. As free traveling is critical for the economy of EU countries. A traditional approach towards travel, including restriction, mandatory testing, and isolation could potentially harm each EU members' benefits. Hence, establishing a convenient and cost-efficient way to manage traveler in the time period of a pandemic was an excellent digital epidemiological method. A side effect that could be easily ignored, but benefits disease control greatly was the impact on vaccine uptake by EU Digital COVID Certificate. As the potential benefits from being vaccinated are reflected on EU Digital COVID Certificate, which further lead to free traveling among EU member states.

However, the EU Digital COVID Certificate is also accompanied by concerns and issues. One big issue around the certificate is the potential discrimination towards vaccine uptake between different states [8]. As different EU member states had different economic development level, the availability of vaccines varies dramatically. Hence those who did not have a quick contact to vaccine might face a potential delay in obtaining the certificates. Also, different states have different policies on vaccine shots and doses, leading to a complex situation where individuals from different member states might have same status on certificate but different vaccination level [7].

Other concerns about the digital certificate are related to fake certificates and data safety [8]. EU law enforcement agency had shown concerns about fake certificates and illegal trading of these certificates among member states [8]. This could be detrimental to the system of digital certificates, as traveling of infected patients is highly risky for the health safety of any communities in EU. Also, fake certificate might negatively affect the trust from citizens towards such centralized digital epidemiological method. Another concern about the certificates is data safety. As the sharing of data with countries outside of EU could lead to the data being used for purposes unrelated to epidemiology or the health fields [8].

4. Application prospective of digital technologies in epidemiology

Newly invented technology and enhanced existing methods are appearing more and more often, they are critical for the adaption of a more digital approach to epidemiology, especially after the pandemic of COVID-19, which have been constantly challenging the current health system. By reviewing the examples of COVIDSafe and EU Digital COVID Certificate, it can be argued that these digital technologies have been serving great roles in combating the spread of virus among individuals, while being truly helpful for epidemiology professionals and authorities. The COVIDSafe application in Australia has offered valuable experiences in the employment of tracking apps within a relatively broad population base. It successfully guarded the health of public by notifying the individual and health authorities about close contacts in a time-saving and cost-efficient fashion. However, we also need to consider its flaws, which includes the suboptimal uptake rate by citizen [4]. EU Digital

COVID Certificate had a different role in mainly contributing to the opening of free travel within EU. While being successful in achieving a safer cross-border traveling experience, its flaws in potential vaccine and resource discrimination and data safety remained [7, 8].

Learning from the experiences discussed above is the key in developing and launching better digital epidemiological strategies in the future. As more people realize the importance of digital epidemiology, such mistakes made previously in the COVID-19 pandemic would be crucial for authorities to re-evaluate their decisions and hopefully invest more on digital epidemiological approach.

5. Conclusion

In conclusion, this paper has given some insights on the basic of digital epidemiology and digital technologies regarding epidemiological issues. By evaluating two distinct but important digital epidemiology application cases in the COVID-19 pandemic: COVIDSafe App and EU Digital COVID Certificate, the author hopes to showcase the importance of digital approach towards health threats like the COVID-19 virus. These case studies shed lights on the potential usage of technologies and data in fighting a global health pandemic. The field of epidemiology will inevitably evolve in order to respond to the changing landscape of diseases and other health threats. There are likely to be more digital strategies being employed in the field of epidemiology in order to prepare us for a more challenging world of public health. One key point that needs to be addressed by the global communities is the equal access to digital epidemiological tools. Technology needs to be vastly available to the public to offer its full potential, and to be an irreplaceable tool in guarding us from health threats in the years to come.

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The influenza viruses in China: Pandemic and prevention measures

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Abstract. Seasonal influenza (SI) is a kind of respiratory virus with annual epidemic and high contagion, leading to cause increased morbidity and mortality in certain population. According to estimates, the flu causes an average of 400,000 respiratory deaths per year worldwide. The estimate mortality of flu is more than 65 per 100,000 people in the worldwide, and the ratio is 52.9/100,000 in China, demonstrating that seasonal influenza has caused a considerable disease burden in China. Influenza viruses are spread by droplets, and people infected with seasonal influenza usually have a sudden high fever, dry cough, and severe headache. Noteworthy, children and gravidas have higher rates of infection, and the elder population over 65 ages has a higher risks for complications, hospitalizations and death, especially patients with cardiovascular and pulmonary diseases. Therefore, prevention and treatment of high-risk populations is important to prevent and control influenza, while vaccination is an effective solution to protect them from infection and associated complications. In this paper, the author analyzes the epidemic features of influenza viruses in different regions of China in light of the prevention and control policies in various places, and provides some suggestions to prevent pandemic of influenza virus and enhance individual's preventive awareness, which purpose is to reduce the influenza positivity rate and the infection rate in the future, especially for vulnerable population of children and elder people.

Keywords: Seasonal Influenza, Epidemic, Prevention, Vaccine.

1. Introduction

Seasonal influenza (SI) is a kind of respiratory virus with annual epidemic and high contagion, leading to cause increased morbidity and mortality in certain population, especially in occasional pandemics. Influenza viruses include strains A, B, C, and D, and strain A virus, as the best known influenza virus, triggered the flu pandemic in 2009. Strain B viruses have less frequent antigenic variations in the genome, limiting themselves to break out and become seasonal influenza and epidemics rarely. Strain C viruses are antigenically stable and do not cause epidemics [1]. There are fewer studies related to influenza D viruses and further research is still needed.

At the end of 2019, novel coronaviruses are spreading around the world, causing a great global health burden. Since Influenza and SARS-CoV-2 share similar transmission pathway, epidemiologists are of the opinion that public health measures taken to prevent the spread of coronavirus also prevented the spread of influenza [2]. Nevertheless, even though the preventive policies during the New Crown period

have been effective in controlling the rate of influenza infection, there are still many problems with influenza prevention and control in China, such as the low rate of influenza vaccination, and the lack of attention to seasonal influenza by the nationals, and so on. In this paper, the author analyzes the epidemic features of influenza viruses in different regions of China in light of the prevention and control policies in various places, and provides some suggestions to prevent pandemic of influenza virus and enhance individual's preventive awareness, which purpose is to reduce the influenza positivity rate and the infection rate in the future.

2. Epidemic characteristics of influenza viruses

Influenza virus mainly break out in winter at temperate zone, while it occurs at various times throughout the year in low latitude with high temperature locations. According to estimates, the flu causes an average of 400,000 respiratory deaths per year worldwide. The estimate mortality of flu is more than 65 per 100,000 people in the worldwide, and the ratio is 52.9/100,000 in China, demonstrating that seasonal influenza has caused a considerable disease burden globally [3].

Influenza viruses are spread by droplets, and people infected with seasonal influenza usually have a sudden high fever, dry cough, and severe headache. The majority of individuals recover within a week without severe clinical signs, sequelae or patient death. Noteworthy, children and gravidas have higher rates of infection, and the elder population over 65 ages has a higher risks for complications, hospitalizations and death, especially patients with cardiovascular and pulmonary diseases [4]. Therefore, prevention and treatment of high-risk populations is important to prevent and control influenza, while vaccination is an effective solution to protect them from infection and associated complications.

Although the disease cycle is short and generally does not lead to serious consequences, the highly contagious and mutable nature of influenza viruses still requires vigilance. As reported in a review study, the median basic reproduction number was 1.80 for the 1918 pandemic, 1.65 for the 1957 pandemic, 1.80 for the 1968 pandemic, 1.46 for the 2009 pandemic, and 1.28 for seasonal influenza [5]. As it spreads, influenza viruses accumulate mutations and then evolve through antigenic drift and antigenic transfer. Based on the above characteristics, epidemiologists suggest that it's necessary to stop transmission between one-third and one-half of infected population through public health measures, thus resulting in the reproductive number lower than 1, eventually leading to epidemic of this virus [5].

3. Epidemic characteristics of influenza viruses in China

Recently, the incidence and serious complications of seasonal influenza has been reduced since the China government improved the public health system. Nonetheless, the spread of influenza viruses has resulted in a high economic burden of disease. The data from national statistics showed that there are 2.5 cases of influenza-like illness (ILI) diagnosed per 1000 individuals annually, and the total number of influenza-associated excess respiratory deaths is between 84,200 and 92,000 every year [6].

The obvious feature of influenza virus epidemics in China is regional differences, which is clearer to display their differences using high and low latitudes. In the high-latitude provinces, the epidemics are predominantly short and intense in the winter of each year, while in the mid-latitude and low-latitude provinces, the epidemics are known for their biannual outbreaks or year-round activity.

For example, the data from the Shihezi hospital from 2010 to 2021, which located in north China, the epidemic of influenza viruses mainly occurred in winter [7], due to the low temperatures. About ILI population, the highest population is aged 5-25 years and the lowest in the age group of 0-5 years [7] because of dramatic discrimination of vaccination and outdoor activity. Another example of hospital in Shanghai from 2010-2019, located in south China, the influenza broke out in winter and summer [8]. The reason of this difference is that high population mobility during the summer and winter tourist peaks increased the risk of virus transmission besides locations. Similar to the hospital in Shanghai, the data from hospitals in Guangdong and Hubei provinces during the period of 2018 to 2020, located far south China, there was a clear winter-spring epidemic peak and occasional summer epidemics of ILI% in the southern region [9, 10]. This phenomenon is related to the humid climate of the southern region is more

suitable for the multiplication of the virus in winter and spring, while the summer is mainly influenced by the lifestyle of southern residents.

Another feature is the main strain virus in both the southern and northern regions, alternating strain A viruses dominated the epidemic from 2018 to 2020, to influenza B viruses emerging as the epidemiologically dominant strain from 2021 (Table 1) [7, 8, 10].

4. Prevention measures of influenza viruses in China

A survey showed that strengthen the awareness, attitudes and knowledge are benefit to prevent and control influenza in south China. Therefore, there are some measures to prevent and control the pandemic of influenza virus, such as strengthen on prevention and control in densely populated places and high-risk groups, improvement of hygiene management in densely populated places, strictly disinfection environments with high population flow, implement random testing for early detection of patients with influenza, advocacy knowledge of influenza prevention and control awareness among exposed populations, and encouragement of adolescents and young adults to receive timely vaccinations to establish an immune barrier. Secondly, the sentinel hospitals are requested to conduct future surveillance by the definition of ILI and specimen collection requirements, and at the same time do a good job of sampling all age groups, especially the young children group and the high age group of over 60 years old [11].

In the northern region, a MEM model was proposed based on determining the baseline of influenza, and an LSTM model was established based on regional and demographic characteristics, while at the same time, a DLNM model was established to find the association between influenza and meteorological factors [12]. The government set up sentinel hospitals and applied these three models to monitor the ILI situation in real-time, and then uploaded them to the China Influenza Surveillance System promptly to provide theoretical support for influenza prevention and control efforts.

By comparing the epidemic prevention policies of the South and the North, it can be found that the influenza prevention focuses on group in south China through addressing the significance of prevention to raise the awareness of prevention, promoting ratio of vaccination to enhance the immunity of the population, eventually achieve the purpose of lowering the infection rate of the population and controlling the spread of diseases. In north China, the prevention measures are be taken in advance of the spread of the virus through monitoring on meteorological factors, studying patterns of climate change during influenza outbreak periods, and analyzing and predicting the epidemic models using software.

What is certain is that China governments are learning lessons and experiences to deal with the epidemic of influenza virus from the 2019 New Crown Pneumonia epidemic [13], such as establishment of multi-surveillance systems, strengthen on influenza control and prevention among high-risk groups and in high-density population centers, as well as enhancement vaccination rate, especially focusing on novel vaccines development with the broad-spectrum and immunogenicity [4]. The introduction and implementation of a variety of measures have provided a fresh boost and positive effect in responding to the challenges posed by post-pandemic influenza.

It is worth noting that, an epidemic rebound usually occurs after a wave of epidemic outbreaks, due to being influenced by climatic conditions, viral antigenic evolution, and host (e.g. human) contact pattern. Individuals vaccination is effective in reducing the rate of infection and morbidity of seasonal influenza, especially for high risk population of children and elder people. At the same time, due to the high mutability of influenza viruses, the updating and applicability of vaccine development are also issues that need to be considered [13]. Besides, it is necessary to choose appropriate vaccines for different groups of people because some vaccines are applicable to most people and are suitable for short-term emergencies during pandemic outbreaks and some vaccines are more suitable for boosting the immune response of naïve people such as young people and children. Special adjuvants may even need to be added to enhance the effects of the vaccines for population with weakened immunity, such as the elderly [4].

In addition, drawing on the lessons learned and experience of the New Crown pandemic, real-time influenza surveillance to ensure that front-line healthcare workers are not infected, is crucial to ensure that hospitals have sufficient resources and strategies in place to meet the demand for additional healthcare resources [2]. Moreover, it is known that during the New Crown Epidemic period, the health code, with its practicality and convenience, made a great contribution to epidemic prevention and control, which can be applied to the monitoring of seasonal influenza. However, solutions need to be prepared in advance before being put into formal application due to the possible lack of credibility in filling in personnel information (Table 1).

Table 1. Epidemic characteristics of influenza viruses in China.

Area	Epidemic Characteristic	Countermeasures	Hidden Problems
North China	Mainly occurs in winter [7]	Epidemiological models analyzed and predicted by relevant agencies using software to develop epidemic prevention policies in real time [12]	Population lacks awareness of prevention to respond to flu in a timely manner
South China	Usually spreads in winter and summer [8]	Medical institutions establish herd immunity by popularizing vaccination, propagating knowledge of epidemic prevention [11]	Population immunization without vaccination policy support is less effective for influenza epidemic rebound
Far south China	Spreads in winter and spring, with occasional summer outbreaks [9]		

5. Conclusion

Seasonal influenza is an annual respiratory virus with highly contagious, which break out threaten health and life in some vulnerable population with higher morbidity and mortality. Therefore, a systematic monitoring system should be established to prevent the epidemic of influenza virus with government support. The education of prevention and vaccination should be carried out a population-based basis, with special protection strategies for high-risk groups. Improving the overall preventive awareness of the population and adherence to epidemic prevention policies can ensure the overall prevention and control of influenza, reduce the disease burden caused by influenza in China, strengthen the national physique, and help accomplish the ambitious goal of Healthy China 2030.

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Comparison of dimensionality reduction methods and evaluation of prediction effect based on cardiovascular disease data

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Abstract. Cardiovascular diseases have become the leading cause of death in the world, and the mortality rate caused by them is still on the rise, which is a major challenge facing the world. In the study of cardiovascular diseases, there are many kinds and quantities of factors leading to the disease, so how to screen more effective factors for research and accurate prediction is an important problem. The aim of this study is to conduct an in-depth comparative analysis of multiple dimensionality reduction methods for cardiovascular disease data. This paper evaluates the results produced by various dimensionality reduction methods and models, and uses Accuracy Rate, Confusion Matrix and Area Under Curve (AUC) and other indicators to evaluate their prediction effects. The study finds that the decision tree model has the best performance and is superior to LDA linear discriminant analysis in terms of accuracy and data dimension. The principal component analysis method is relatively complex, although it can effectively reduce the data dimension, its accuracy in forecasting decreases, and this method is not conducive to horizontal and vertical comparison and the accumulation of statistical data.

Keywords: Cardiovascular diseases, dimensionality reduction methods, prediction, accuracy, data dimensions.

1. Introduction

Cardiovascular diseases (CVDs), as the world's leading fatal diseases [1], have shown a sharp increase in recent years [2], and are a major global challenge to public health. Effective preventive measures against cardiovascular diseases are essential [3], and predicting the occurrence of cardiovascular diseases can provide great theoretical significance and applied value for the treatment of clinical cardiovascular diseases [4]. At present, in the medical field, facing the identification, diagnosis and detection of complex problems such as cardiovascular diseases, the use of dimensionality reduction methods to deal with the relevant datasets is conducive to the elimination of research interfering information, which in turn helps to predict the relevant problems [5].

In order to better understand and predict the occurrence of cardiovascular diseases, various data-driven methods have been applied to data containing many complex features. Wang and others used the Join point regression model to analyse the average annual percentage change (AAPC) of cardiovascular disease (CVD) mortality in the elderly in China, and applied the GM(1,1) model to predict the crude

rate of CVD deaths among the elderly in China from 2020 to 2030, pointing out that males are higher than females, and rural areas are higher than urban areas, and predicting that the crude rate of CVD deaths among the elderly in China will decline year by year, but still remain at a high level[3]. Liu used the use of survival analysis method of cardiovascular disease risk factors for single-factor analysis, and the use of Cox proportional risk model to carry out risk factors and the risk of morbidity of multifactorial analysis. At the same time, a prediction model for cardiovascular morbidity risk factors was established, pointing out that the risk factors in the problem of synergistic effect between each other, not only to pay attention to the number of risk, but also to pay attention to the combination between different risk factors [6]. Malovini et al. pointed out that because some traits are the result of a combination of rare and common variants and non-genetic factors, multivariate methods can help to analyse the genetic characteristics of cardiovascular diseases by compensating to some extent for informative genetic variants that had been overlooked by traditional univariate methods [7]. According to the Coronary Heart Disease (CHD) formula, Anderson K M and others showed that it is potentially important to control multiple risk factors instead of focusing one single risk factor in the studies related to cardiovascular disease. Parametric Model is better than some existing standard regression models. It can predict different time lengths and its probability expression is more direct than Cox proportional risk model [8]. Based on public health data, scholar Cui built a dynamic risk prediction model of cardiovascular disease using a longitudinal submodel connected by association structure and a Multivariate joint model of survival submodel, and obtained predictive indicators of the risk of cardiovascular disease in men and women. It was also pointed out that the area under the curve (AUC value) of the prediction model constructed by this model method is higher than that of the prediction model constructed by the multi-factor Cox proportional risk regression model method, confirming that this method is superior to the Cox proportional risk regression model [9]. VanDer et al. pointed out that the dimensionality reduction methods remove redundant and irrelevant features through feature extraction, and convert the original dataset containing high dimensional data (HDD) into a new dimensionality reduction dataset [10]. However, different dimensionality reduction techniques (DRTS) can produce significantly different results. Therefore, it is particularly important to choose the best dimensionality reduction method based on a given data set and analysis task. Ayesha S and others believed that the variation of results may come from differences in basic assumptions, mathematical formulas, and adaptability to data features [11].

Therefore, this study applies a variety of dimensionality reduction methods to the cardiovascular disease data set, and evaluates the results produced by these methods to interpret the reasons behind the observed differences in results, using the comparison of their AUC value, Accuracy Rate, Confusion Matrix and other indicators.

2. Methods

2.1. Data source and description

The data set used in this paper comes from KAGGLE website, with about 70,000 pieces of data. The variables and forms of the data set are shown in Table 1, including 11 independent variables and 1 target variable. The data set is of high quality and suitable for the subsequent research methods of this paper.

Table 1. Data description.

Variable	Symbol	Characteristic	Unit
Age	age	Objective Feature	days
Height	height	Objective Feature	cm
Weight	weight	Objective Feature	kg
Biological Gender	gender	Objective Feature	1-Female;2-Male
Systolic Blood Pressure	ap_hi	Examination Feature	mmHg
Diastolic Blood Pressure	ap_lo	Examination Feature	mmHg

Table 1. (continued).

Cholesterol	cholesterol	Examination Feature	1-normal 2-above normal 3-well above normal
Glucose	gluc	Examination Feature	1-normal 2-above normal 3-wel above normal
Smoking	smoke	Subjective Feature	0-no;1-yes
Alcohol Intake	alco	Subjective Feature	0-no;1-yes
Physical Activity	active	Subjective Feature	0-no;1-yes
Presence of CVDs	cardio	Target Variable	0-no;1-yes

2.2. Index selection and description

This study will first introduce the dimensionality reduction methods under consideration, including principal component analysis (PCA) in linear dimensionality reduction, linear discriminant analysis (LDA), and decision tree model. Principal component analysis (PCA) is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss [12]. Linear discriminant analysis (LDA) is the most widely used supervised dimensionality reduction approach. After removing the null space of the total scatter matrix S_t via principal component analysis (PCA), the LDA algorithm can avoid the small sample size problem [13]. Decision tree classification algorithm is one of the most widely studied and applied topics in data mining, with high application value. However, this method may over-fit training data and be easily affected by noisy data, so the application of pruning method is very important [14].

2.3. Method introduction

In this study, the missing data and abnormal data will be reasonably filled and cleaned to meet the prerequisite requirements of different dimensionality reduction methods. Various dimensionality reduction techniques mentioned above will be applied to the pre-processed data set to produce dimensionality reduction results.

Secondly, this study will display the dataset qualitatively and quantitatively. Qualitative analysis includes data visualizations, such as boxplots and bar charts, to identify how is the data distributed. More in-depth quantitative analysis will be used to identify differences in capture variance and feature discrimination. In order to analyze the reasons for the observed differences in results, this study will conduct an in-depth investigation, including the inherent assumptions, mathematical foundations, and algorithmic procedures of each dimensionality reduction method, and reference the relevant literature to understand the nuances that contribute to the differences in results.

Finally, in order to quantitatively evaluate the performance of each dimensionality reduction method, this study adopts a series of indicators, including accuracy, confusion matrix, receiver operating characteristic curve (ROC) and the values of Area Under Curve (AUC). In the calculation of the above indicators, appropriate cross-validation strategies are adopted to ensure robustness and generalization.

By using the above methods, this study aims to comprehensively and systematically analyze the effects of various dimensionality reduction methods on the analysis of CVD data sets. By comparing results, exploring the causes of differences, and evaluating quantitative performance, it will help to deepen the understanding of the utility of different techniques in enhancing cardiovascular disease prediction.

3. Results and discussions

3.1. Data preprocessing

There are 70,000 data in the initial dataset. Figure 1 shows the situation of cardiovascular diseases of different genders. It can be seen that among the men involved, the number of people whether suffering

from cardiovascular diseases are both about 23,000, and the number of women related to those are about 12,500. It can be preliminarily determined that gender has a small effect on the risk of cardiovascular disease.

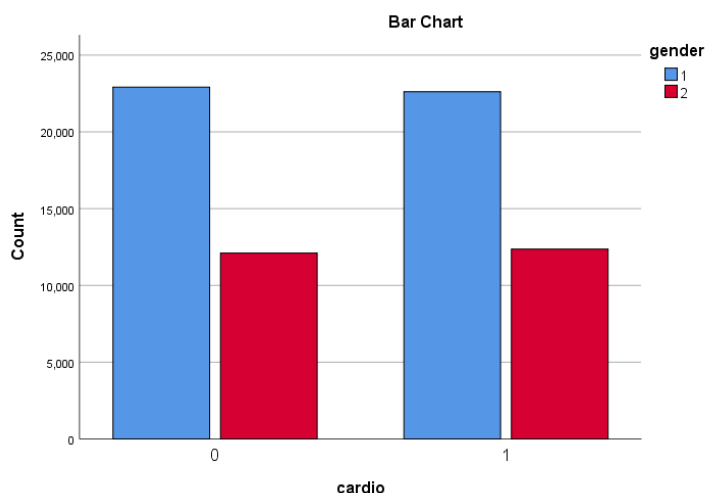


Figure 1. Gender bar chart.

As shown in Figure 2, the average age of people without cardiovascular disease is about 19,000 days, while the other is about 20,500 days, which shows that the latter is higher than the former. It can be preliminarily inferred that cardiovascular disease is more common in people with higher age.

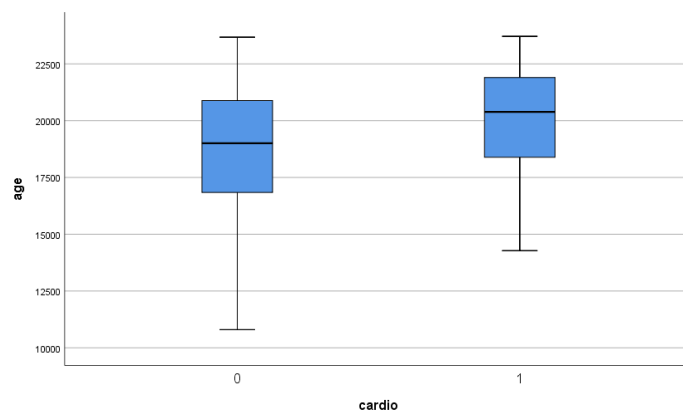


Figure 2. Age boxplots.

In this study, the values of age, height, weight, systolic blood pressure and diastolic blood pressure were standardized. Different normal value ranges were selected for different variables. Abnormal values were eliminated according to this range, leaving 66,753 valid data.

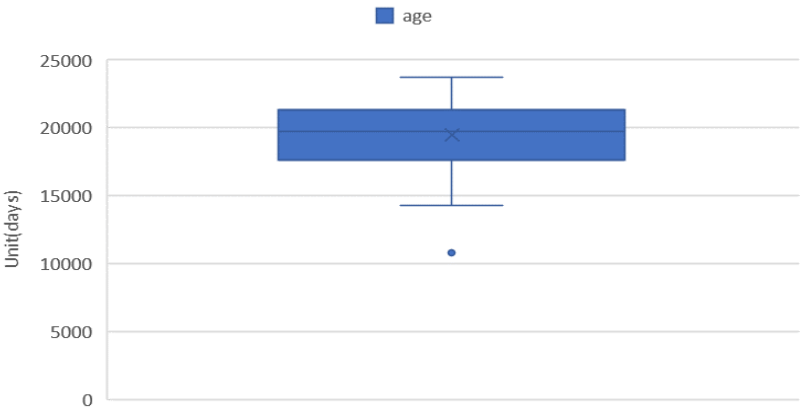


Figure 3. Adjusted age boxplots.

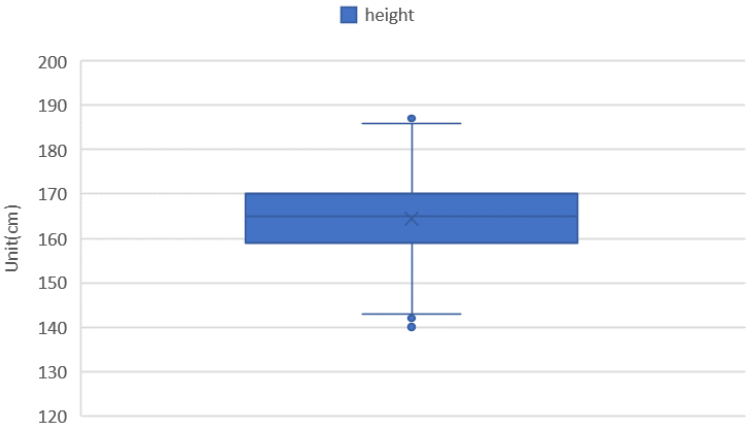


Figure 4. Adjusted height boxplots.

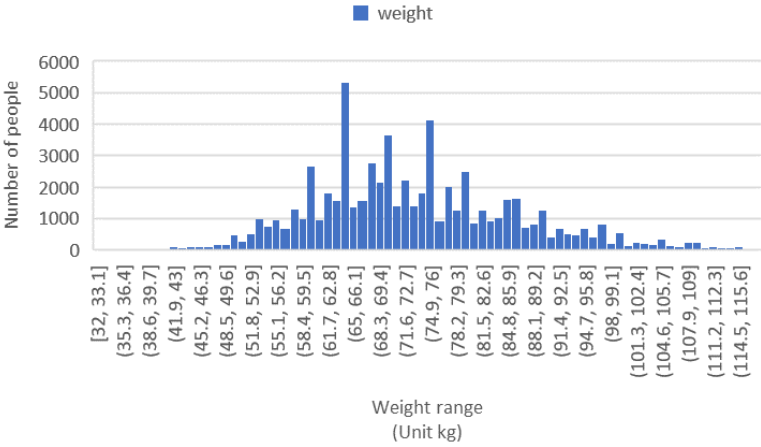


Figure 5. Adjusted weight histograms.

After adjustment, there is no extreme value greater than 3 times the box distance in the data set, but there is an outlier value greater than 1.5 times the box distance. Figure 3, 4 and 5 show the current characteristics of the new data set. For the values of age, height and weight, it can be concluded that these outliers can be retained. Therefore, there are no outliers that need to be processed, and this data

set can be used to carry out follow-up research. Table 4 show the descriptive statistics of 11 independent variables and 1 dependent variable in the adjusted data set. It can be seen from Table 2 that compared with the data before adjustment, the variables such as systolic blood pressure and diastolic blood pressure in the processed data set are in the normal range, and the overall data quality is higher.

Table 2. Descriptive statistics.

	N	Minimum	Maximum	Mean	Std. Deviation
age	66753	10798	23713	19454.90	2469.135
gender	66753	1	2	1.35	.476
height	66753	140	188	164.38	7.675
weight	66753	32.00	117.00	73.4562	13.14647
ap_hi	66753	80	170	125.71	15.160
ap_lo	66753	50	111	80.94	8.913
cholesterol	66753	1	3	1.36	.674
gluc	66753	1	3	1.22	.567
smoke	66753	0	1	.09	.282
alco	66753	0	1	.05	.223
active	66753	0	1	.80	.397
cardio	66753	0	1	.49	.500
Valid N (listwise)	66753				

The data set was then divided into a training set and a test set in a ratio of 8:2.

3.2. Dimensionality reduction technique

3.2.1. PCA principal component analysis. PCA principal component analysis maps M-dimensional features to N-dimensional features, which are new orthogonal features, also known as principal components. It should be noted that each principal component is a linear combination of all the original variables, and each principal component is not related to each other. The principal components obtained by this method are more representative than the original independent variables.

After standardizing the data, the correlation matrix and total variance interpretation are obtained by calculating covariance, eigenvalue, information contribution rate and cumulative contribution rate. Then the principal component function is obtained by calculating the coefficients of each original variable standardized in each principal component, and then the principal component is determined. The weight of each principal component is obtained through the variance percentage of the sum of squares of loads extracted from the total variance interpretation table. After normalization, the equation for calculating the comprehensive score of each data can be obtained, and then the comprehensive evaluation score can be obtained.

Table 3. Total Variance Explained.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.199	19.990	19.990	2.199	19.990	19.990
2	1.883	17.120	37.110	1.883	17.120	37.110
3	1.305	11.862	48.973	1.305	11.862	48.973
4	1.114	10.129	59.102	1.114	10.129	59.102

Table 3. (continued).

5	.991	9.011	68.113	.991	9.011	68.113
6	.893	8.118	76.231	.893	8.118	76.231
7	.771	7.010	83.241	.771	7.010	83.241

Table 4. Component Matrix^a.

	Component						
	1	2	3	4	5	6	7
ap_hi	.732	-.355	-.367	.169	-.015	-.114	-.137
ap_lo	.713	-.319	-.405	.168	-.012	-.176	-.172
gender	.425	.652	.027	-.157	.033	.217	-.343
height	.393	.630	-.036	-.451	.099	.066	-.031
smoke	.307	.539	.236	.442	-.134	.035	-.156
gluc	.297	-.324	.702	-.194	.024	-.104	-.147
cholesterol	.401	-.397	.615	-.078	.030	-.074	-.061
alco	.250	.374	.266	.603	-.179	-.101	.388
active	.003	.029	.045	.282	.956	.059	.021
age	.280	-.361	.011	.075	-.081	.865	.157
weight	.580	.102	-.064	-.377	.092	-.133	.615

On the basis of having passed the significance test, the following is used to illustrate the parameters. Table 3 shows the extraction of the square sum of variance percentage of load, and the principal component analysis method is used to extract 7 principal components from the original 11 independent variables, and the proportion of these principal components in explaining the results is 83.241%. Meanwhile, Table 4 shows the factor load matrix. The following is an explanation of these 7 principal components, which are Y_n ($n = 1, 2, \dots, 7$).

$$Y_1 = 0.494 \times Zap_{hi} + 0.481 \times Zap_{lo} + \dots + 0.391 \times Zweight \quad (1)$$

Y is the comprehensive score of each piece of data. The weight of each principal component is known by extracting the square of load and the percentage of variance, divided by the cumulative contribution rate. After normalization of these weights, the expression of the comprehensive evaluation score can be obtained.

$$Y = \frac{(0.19990 \times Y_1 + 0.17120 \times Y_2 + 0.11862 \times Y_3 + 0.10129 \times Y_4 + 0.09011 \times Y_5 + 0.08118 \times Y_6 + 0.07010 \times Y_7)}{0.83241} \quad (2)$$

Finally, let the new data set obtained by principal component analysis be dataset 1.

3.2.2. LDA linear discriminant analysis. When training samples, LDA linear discriminant analysis needs to find a straight line and project the training samples to a line that can make the projection points of the same type of samples as close as possible, while the projection points of different types of samples are as far away as possible. During the prediction, the data to be predicted is projected onto the line, the category is determined according to the position of the projection point, and the prediction effect is evaluated by the accuracy of the prediction, so as to test whether the current independent variable can explain the corresponding results, and then determine the valuable dimensionality reduction data set.

Table 5. Variables Entered/Removed

Step	Entered	Wilks' Lambda							
		Statistic	df1	df2	df3	Statistic	df1	df2	Sig.
1	ap_hi	.811	1	1	53400.000	12454.790	1	53400.000	.000
2	age	.788	2	1	53400.000	7180.993	2	53399.000	.000
3	cholesterol	.773	3	1	53400.000	5227.104	3	53398.000	.000
4	weight	.770	4	1	53400.000	3983.362	4	53397.000	.000
5	active	.769	5	1	53400.000	3211.392	5	53396.000	.000
6	ap_lo	.768	6	1	53400.000	2684.717	6	53395.000	.000
7	smoke	.768	7	1	53400.000	2307.908	7	53394.000	.000
8	gluc	.767	8	1	53400.000	2023.961	8	53393.000	.000
9	height	.767	9	1	53400.000	1801.808	9	53392.000	.000
10	alco	.767	10	1	53400.000	1623.878	10	53391.000	.000

As can be seen from Table 5, an independent variable named 'gender' is eliminated by this method, and the significance of the remaining independent variables is 0.00, so there are 10 remaining factors. At the same time, the discriminant equation has passed the validity test, and its significance is 0.00, which can be considered that the discriminant ability of the equation is significant.

Table 6. Standardized Canonical Discriminant Function Coefficients.

	Function
	1
age	.314
height	-.042
weight	.143
ap_hi	.729
ap_lo	.078
cholesterol	.300
gluc	-.053
smoke	-.034
alco	-.040
active	-.087

A standardized typical discriminant equation expression can be obtained from Table 6, that is $Y = 0.314x_1 - 0.042x_2 + 0.143x_3 + 0.729x_4 + 0.078x_5 + 0.3x_6 - 0.053x_7 - 0.034x_8 - 0.04x_9 - 0.087x_{10}$. Through this method, 72.6% of originally grouped cases were correctly classified. Finally, let the new dataset obtained by LDA linear discriminant analysis be dataset 2.

3.2.3. Decision tree model. The Chi square automatic interaction detection (CHAID) decision tree algorithm optimally segments the sample according to the given target variable and explanatory variable, performs automatic judgment grouping of multivariate contingences according to the significance of Chi-square test, and divides the dataset into the subset with the least impurity until the stopping criterion is met.

The original training data set was divided into the training set and the test set of the decision tree model at a ratio of 7:3 to verify the explanatory degree of the extracted new independent variable to the dependent variable. The growth method of CHAID can be used to quickly and efficiently mine the main influencing factors, that is, to select the best attribute and segmentation point and judge the priority of the attribute by calculating the Chi-square statistic between each variable and the target variable. The chi square statistic measures the correlation between the two variables, and the variable with a larger Chi-square value has a higher correlation with the target variable. After calculating the Chi-square value corresponding to the target attribute and each attribute, the optimal attribute is found according to the classification of the parent node, and the first level of the decision tree is obtained.

Table 7. Model Summary.

	Growing Method	CHAID
	Dependent Variable	cardio
Specifications	Independent Variables	age, gender, height, weight, ap_hi, ap_lo, cholesterol, gluc, smoke, alco, active
	Validation	Split Sample
	Maximum Tree Depth	3
	Independent Variables Included	ap_hi, age, weight, cholesterol, active, gluc
Results	Number of Nodes	70
	Number of Terminal Nodes	46
	Depth	3

As can be seen from Table 7, the original 11 independent variables can be analyzed through the decision tree model to determine whether there is cardiovascular disease, and 6 independent variables (systolic blood pressure, age, weight, cholesterol, physical activity and glucose) can be analyzed, and the result is obtained, the depth of the decision tree model is 3.

Table 8. Risk.

Sample	Estimate	Std. Error
Training	.185	.001
Test	.188	.002

According to the risk table, as shown in Table 8, the training estimate is 0.185, and 18.5% of the 70% training samples are misclassified. The test estimate was 0.188, with 18.8% of the 30% tested samples misclassified. It can be seen that the decision tree model does not overfit when more than 80% of the correct rate is guaranteed, indicating that the model has certain significance. Finally, let the new dataset obtained from the decision tree model be dataset 3.

3.3. Comparison of dimensionality reduction methods

When the dimensionality reduction results obtained by principal component analysis are used for prediction, the output variable is the comprehensive score, which cannot be unified with the dependent variable 'cardio'. Therefore, this study uses the dimensionality reduction data set obtained by principal component analysis to conduct logistic regression and obtain the predicted 'cardio' variable, which can be compared equally with other dimensionality reduction methods.

First of all, from the above, we can get the characteristics of the explanatory variables in the data set processed by each method and model. In the dimensionality reduction data set obtained by principal component analysis, there are 7 explanatory variables. After combining with the forward stepwise regression analysis of logistic regression, the variables are filtered, so there are 6 explanatory variables, which are linear combinations formed by the original independent variables. Second, when the forward

stepwise regression is performed alone, the independent variable 'gender' is not included in the prediction equation. Furthermore, the independent variable 'gender' was also not included after the LDA linear discriminant analysis, which is the same as the data set obtained when the logistic regression was performed alone. Although LDA linear discriminant analysis and logistic regression are similar in form, their assumptions, parameter estimation methods and model meanings are different. Finally, the decision tree model is used to classify the six main independent variables, and the number of explanatory variables generated by this model is the least in the dataset obtained by the dimensionality reduction methods in question.

Secondly, in terms of fit degree, after principal component analysis combined with logistic regression, the overall percentage of correct prediction of both the training set and the test set was 72%. The accuracy of the LDA linear discriminant analysis training set is 72.6%, and its accuracy is also close to the accuracy of the training set when using the test set for verification, but the difference between them is slightly increased compared with the first method. A 7:3 ratio of training set and validation set was adopted when the decision tree model was constructed, and the misclassification ratio of the two are almost the same, 18.5% and 18.8% respectively. Therefore, neither the dimensionality reduction methods nor the prediction models have overfitting.

Then, the ROC curve and its area under the curve, AUC, were used to evaluate the prediction effects of the above methods and models. In this study, the following four dimensionality reduction methods and prediction models were used to compare with the actual value of the 'cardio' variable of the original training set. They were respectively principal component analysis method combined with forward stepwise regression, and directly carried out forward stepwise regression of logistic regression, LDA linear discriminant analysis method and decision tree model on the original training data set, and then drew the ROC curve. The AUC value is obtained by calculating the area under the curve.

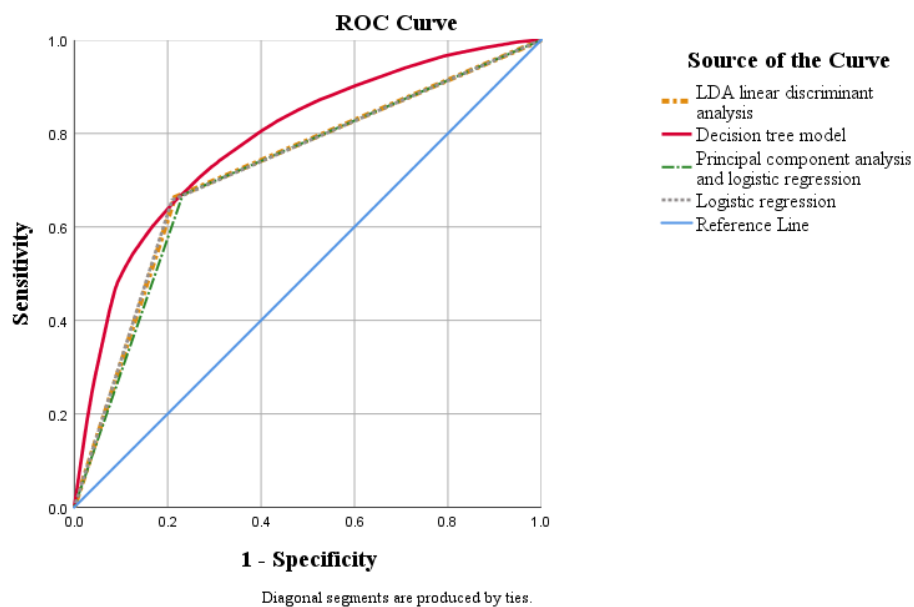


Figure 6. ROC Curve.

When the ROC curve as a whole is closer to the point (0,1), that is, the closer it is to the top left of the coordinate system, the corresponding method or model is better. Figure 6 shows the ROC curve. The intersection point of these four methods and the ROC curve of the model is roughly (0.22,0.65). Among them, the decision tree model has the best effect, and the other three kinds of prediction effect are very close. It is worth noting that the prediction effect of logistic regression alone is better than that of principal component analysis combined with logistic regression, and the difference between the two is not large. It also indirectly reflects the relationship of explanatory variables in the dimensionality

reduction dataset obtained by LDA linear discriminant analysis and forward stepwise regression of logistic regression.

Table 9. Area Under the Curve.

Test Result Variable(s)	Area	Std. Error ^a	Sig.	Asymptotic 95% Lower Bound	Confidence Interval Upper Bound
LDA linear discriminant analysis	.724	.002	.000	.720	.728
Decision tree model	.788	.002	.000	.784	.792
Principal component analysis and logistic regression	.718	.002	.000	.714	.723
Logistic regression	.724	.002	.000	.720	.729

a.Null hypothesis: true area = 0.5

Table 9 describes the AUC indexes of the above four methods and models. In this study, the AUC values of LDA linear discriminant analysis, decision tree model, principal component analysis combined with forward stepwise regression and separately forward stepwise regression are 0.724, 0.788, 0.718 and 0.724 respectively. Therefore, the diagnosis accuracy of decision tree model is higher, followed by LDA linear discriminant analysis and forward stepwise regression, and the lowest is principal component analysis combined with forward stepwise regression, and the AUC values of the four are relatively close. It can be roughly inferred that after dimensionality reduction of principal component analysis, its accuracy is not as high as that of direct logistic regression prediction on the original training data set.

Finally, in terms of computational complexity, the combination of principal component analysis and forward stepwise regression has the highest processing complexity. This method not only does not reduce the number of raw variables to be collected, but also requires linear combination calculation of the original variables, and then it may be necessary to compare the results with other predictive models. LDA linear discriminant analysis and forward stepwise regression require 10 explanatory variables with moderate complexity. After the decision tree model is processed, only 6 explanatory variables are retained, which greatly reduces the indicators to be collected. Moreover, due to the reduction of dimensions, the number of samples to be collected can be reduced to a certain extent, and its complexity is moderate. Therefore, the decision tree model performs better in this respect.

To sum up, the performance of decision tree model is the best, followed by LDA linear discriminant analysis, but its fit degree is relatively low and unstable. Principal component analysis is relatively complex and its prediction performance is relatively weak.

4. Conclusion

For these three methods and models, there are no overfitting cases. Among them, decision tree model has higher accuracy and can greatly simplify the burden of data collection in terms of sample size and data dimension. The complexity of the LDA model is moderate, and the independent variable gender can be omitted in terms of sample requirements, and its accuracy is also high, but the fitting degree may be unstable, and its accuracy is relatively low. Although the principal component analysis method can reduce the data dimension, it also needs to collect the original data of the same dimension, and only after linear combination operation can the data set with lower dimension be obtained. Besides, its accuracy is relatively low, and it requires high manpower and material resources input in the early sampling, and it also has high complexity in the later calculation and analysis. Therefore, this study believes that LDA linear discriminant analysis or decision tree model can be used for data reduction and result prediction when dealing with cardiovascular disease or similar data sets and predicting related results, so as to obtain more efficient processing and more reliable prediction results.

Similarly, by analyzing the characteristics of the three dimensionality reduction datasets obtained, the present study concluded that age, blood pressure (systolic and diastolic), cholesterol, weight,

physical activity, and glucose are seven important measures of cardiovascular disease. Therefore, for cardiovascular disease researchers, they can focus on analyzing and screening the above indicators to preliminarily predict and determine whether someone has the risk of cardiovascular disease. For the general public or healthy people, they can enhance their health awareness based on these seven indicators. For example, with the growth of age, the time interval of physical examination should be appropriately shortened, the possible cardiovascular health problems should be detected early, and the treatment time should be better. They can also control and monitor their own weight and blood pressure, as well as the intake of cholesterol and glucose, and carry out appropriate physical exercise. To help reduce your risk of cardiovascular disease.

This study also has the following deficiencies. First of all, this paper does not subdivide cardiovascular diseases, but only classifies all cardiovascular diseases and takes whether to suffer from cardiovascular diseases as its attribute value. The specificity and pertinence of these diseases need to be improved. Secondly, in order to compare the prediction accuracy, this study combined principal component analysis with logistic regression as a prediction model to compare with other independent methods or models, which may lead to errors caused by different comparison baselines. Therefore, when comparing methods and models, a more suitable uniform comparison baseline should be sought.

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Prevalence of antibiotic resistance in *Clostridium difficile*: A meta-analysis

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Abstract. In recent years, the resistance of *Clostridium difficile* to various common mainstream antibiotics cannot be ignored, the incidence of CDI in many areas is also increasing year by year, posing a great threat to public safety. Literature collection was carried out on 6 open databases, and high-quality literature data conforming to classification criteria were screened and extracted. The WPR and WPF of each antibacterial drug were calculated using the random effects model. Subgroup analysis was used to investigate the influence of regional and experimental methods. Results: A total of 40 studies were included. The WPR of vancomycin was 0.0372, 95%CI: [0.0148; 0.0671], I²: 93.7%, WPF was 0.2195, I²: 94.0%. The WPR of fidaxomicin was 0.0051, 95%CI: [0.0000; 0.0205], I²: 82.7%, WPF was 0.1757, I²: 93.6%. The WPR of metronidazole was 0.0318, 95%CI: [0.0056; 0.0727], I²: 91.6%. Among the three antibiotics, the highest resistance of *C. difficile* to metronidazole were mainly distributed in the Middle East and Africa. The conclusion seems to indicate that *C. difficile* is most sensitive to Fidaxomicin, and the clinical cure rate is better than vancomycin. However, the clinical cure failure rate increased significantly from 2007 to 2019, while the drug resistance rate did not change significantly, suggesting that other factors besides drug resistance may affect the therapeutic effect of fidaxomicin on CDI. There was no significant difference in the results of AGAR dilution and e test, the main methods for drug susceptibility testing.

Keywords: *Clostridium difficile*, antimicrobial resistance, meta-analysis.

1. Introduction

Clostridium difficile (*C. difficile*), a Gram-positive bacterium capable of forming spores [1], was first identified in the anaerobic fecal microbiota of newborns [2]. Initially, Bartlett et al found that *C. difficile* could cause antibiotic-associated pseudomembranous colitis in patients [3]. Weakening of colonization resistance in the gut flora allows unrestricted colonization by *C. difficile*, leading to *C. difficile* infection (CDI) [4]. The misuse of some commonly used broad-spectrum antibiotics, including penicillins and cephalosporins, is the leading cause of CDI.

In recent times, there has been an increasing focus on the issue of microbial antibiotic resistance resulting from the inappropriate use of antibiotics [5], especially the antibiotic resistance of *C. difficile* in the treatment and prevention of CDI. Even though FMT has been validated as a efficacious therapy for recurrent *Clostridium difficile* infection (rCDI) [6], and numerous clinical trials of newly developed

narrow-spectrum antibiotics effective against *C. difficile* have been completed [7, 8], a series of antibiotic regimens, such as vancomycin, fidaxomicin and metronidazole, remain a mainstream and cost-effective method for both initial and recurrent CDI patients today. However, many prevalent clinical isolates of *C. difficile* are multidrug-resistant, with a noticeable increasing trend, posing a significant threat to public safety [9-11]. Furthermore, according to data from the Centers for Disease Control and Prevention (CDC) in the United States in 2015, there were approximately 453,000 cases of CDI annually, resulting in approximately 29,000 fatalities. Since 2002, the global incidence of CDI has shown a consistent year-on-year increase, affecting not only one specific country but various regions across the world [12, 13], making research on *C. difficile* antibiotic resistance particularly important.

In summary, in response to the significant issue of *C. difficile* antibiotic resistance, this paper will collect and screen clinical data on *C. difficile* antibiotic resistance research from publicly available databases between 2010 and 2023. It will conduct a meta-analysis of the resistance results produced by the three antibiotics mentioned above and explore the heterogeneity sources through heterogeneity testing. Subgroup analyses will be conducted to discuss the contributions of variables such as region to heterogeneity, revealing the multidimensional differences in *C. difficile* antibiotic resistance. Using these methods to investigate antibiotic resistance in *C. difficile* under different antibiotics is of great significance for analyzing the risk factors for resistance and developing antibiotics against *C. difficile*. It also holds important reference value for improving existing clinical antibiotic treatment for CDI.

2. Methods

2.1. Data origin and selection

2.1.1. Search strategy. The main data sources for this study are from databases including Wanfang Data, CNKI (China National Knowledge Infrastructure), PubMed, Science of Direct, Web of Science, and Scopus. The search was conducted using random combinations of keywords such as “*Clostridium difficile*”, “antimicrobial agents”, “antibiotics”, “resistance”, “clinical trials”, “vancomycin”, “fidaxomicin”, and “metronidazole”, among others. Articles published from June 2005 to June 2023 were included in the study.

2.1.2. Selection criteria. All studies included in the analysis were screened multiple times by three authors, and were excluded if they had the following conditions: (1) The study did not present antibiotic resistance results for *Clostridium difficile*. (2) The resistance rate was not clearly reported. (3) The study was a meta-analysis, systematic review, or not an original research article. (4) Duplicate reports from the same database. (5) Conference abstracts and articles without full text as requested by the authors. (6) The antibiotics used did not include vancomycin, fidaxomicin, or metronidazole (if one of these was used, it was considered for inclusion).

2.1.3. Definition of antimicrobial resistance. According to guidelines provided by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI), the determination of resistance is typically based on clinical assessment and the minimum inhibitory concentration (MIC) of the microorganism. In this study, following a comprehensive data assessment, a lower limit will be defined as the criterion for ascertaining resistance, thus standardizing the qualitative results (presence or absence of resistance).

2.2. Research protocol

2.2.1. Data extraction. After retrieving the full-text data, each article will be systematically assigned a unique identifier, and a comprehensive set of information will be gathered from each article, including its title, authorship, journal source, publication date, research objectives, study type, geographical focus, and target population. Additionally, details about disease diagnostic criteria and outcome assessment

standards will be collected. The statistical methods employed in the studies, the quality assessment applied, and the reported results and conclusions will also be documented.

2.2.2. Quality assessment. The quality assessment of the research will be conducted in accordance with the quality assessment criteria outlined in the Cochrane Systematic Reviewer's Handbook, which includes evaluating the literature's quality and bias risk. The Revman quality assessment tool will be employed to appraise the methodological quality of the studies that are finally included for analysis. Initial evaluations will be performed by two reviewers, and any discrepancies in the literature data will be adjudicated by a third reviewer.

2.2.3. Statistical analysis. In the R programming software, a meta-analysis will be performed. A forest plot will be created, and the I^2 statistic will be calculated. Utilizing Bayesian Markov Chain Monte Carlo (MCMC) algorithms, covariate-adjusted analyses will be conducted on the data derived from the random-effects model. Efficacy and resistance analysis will utilize rates as the statistical measure, with continuous data measurements relying on weighted mean differences or standardized mean differences. Each effect size will be reported with a 95% confidence interval (CI).

2.2.4. Study outcomes. Subgroup analyses will be conducted mainly based on geographic regions (North America, Europe Union, Asia-Pacific, Middle East and Africa). In case significant heterogeneity is observed and there are more than five studies included, further subgroup analyses will be considered based on publication date, treatment duration, antimicrobial susceptibility testing methods, research quality, among other relevant factors. When the number of studies within a category exceeds five, potential publication bias will also be analyzed.

3. Results and discussion

3.1. Query outcomes

A total of 518 articles published between June 2005 and June 2023 were included following an initial screening of titles and abstracts, subsequent to indexing from six databases. Following full-text review, 424 articles were excluded, and 94 articles underwent a quality assessment (Fig. 1).

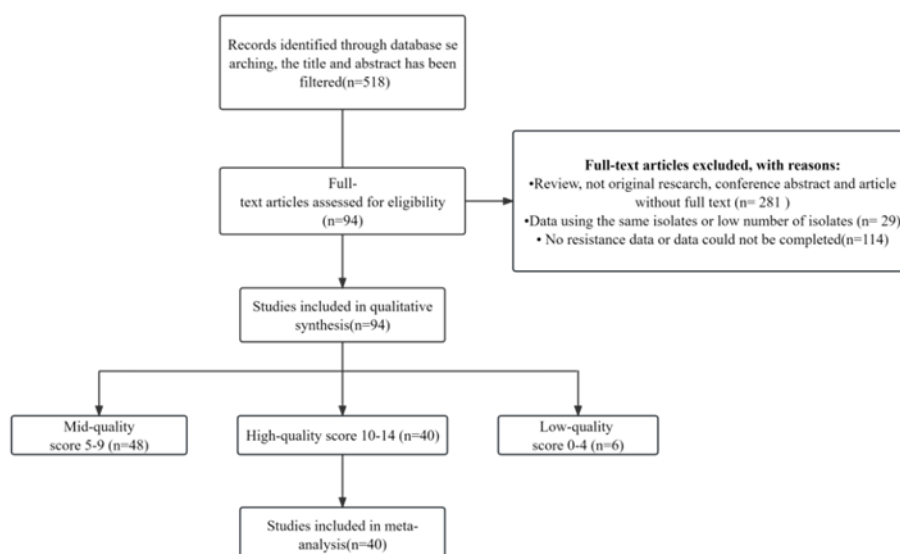


Figure 1. Chart Displaying the Study Screening Progress.

Criteria for non-inclusion during the second screening encompassed the absence of resistance data, non-original research, and unavailability of full-text, among others. Quality assessment scores ranged from 5 to 13 points. Ultimately, 40 articles with scores of 10 or higher and complete data were incorporated (Fig. 1).

3.2. Features of included studies

The 40 studies included in this analysis encompassed randomized controlled trials (RCTs) conducted in a total of eight countries and regions, with a sample size of 7,458 participants (4,840 for vancomycin, 2,722 for metronidazole, and 4,075 for fidaxomicin). The majority of studies originated from the Americas, including North America and South America (n=16), followed by the Asia-Pacific region (n=11). The agar dilution method was the most commonly used antimicrobial susceptibility testing technique (n=24), followed by the E-test method (n=9). All studies employed a cross-sectional design. Importantly, all 40 studies (100%) were considered to be of good quality and with limited bias. For the distribution of publication years, please refer to Table 1, 2, and 3 for specific details regarding the categorization of the three antibiotics.

Table 1. An overview of certain literature integrated into the Vancomycin Meta-Analysis.

author	year	region	test	evaluation	n	n_resistance	MIC_breakpoint µg/ml
Goldstein, E. J. et al.	2011	CA	Agar dilution	10	321	21	2
Goldstein, E. J. et al.	2011	US	Agar dilution	10	285	29	2
Nerandzic, M. M. et al.	2012	NA	Agar dilution	11	301	54	2
Nerandzic, M. M. et al.	2012	NA	Agar dilution	11	141	10	2
Gerding, D. N. et al.	2016	NA	Agar dilution	11	78	1	2
Chen Yunbo	2010	APac	E-test	12	163	0	2
Emmanuel, A. Z. et al.	2015	MX	E-test	11	31	0	4
Emmanuel, A. Z. et al.	2016	MX	E-test	11	63	0	4
Kouhsari, E. et al.	2017	MEA	E-test	12	35	3	2

Table 2. An overview of certain literature integrated into the Fidaxomicin Meta-Analysis.

author	year	region	test	evaluation	n	MIC_breakpoint	treatment_failure_
Goldstein, E. J. et al.	2011	NA+EU	Agar dilution	10	396	1	46
Petrella, L. A. et al.	2012	NA+EU	Agar dilution	11	120	1	15
Mikamo, H. et al.	2018	JP	Agar dilution	12	104	2	34

Table 3. An overview of certain literature integrated into the Metronidazole Meta-Analysis.

author	year	region	test	evaluation	n	n_resistance	MIC_breakpoint t µg/ml
Goldstein, E. J. et al.	2011	CA	Agar dilution	10	321	21	2
Goldstein, E. J. et al.	2011	US	Agar dilution	10	285	29	2
Goldstein, E. J. et al.	2011	EU	Agar dilution	10	119	17	2
Emmanuel, A. Z., et al	2015	MX	E-test	11	31	0	4
Emmanuel, A. Z. et al	2016	MX	E-test	11	63	0	4
P Shoaiei. et al	2016	MEA	E-test	11	19	0	2
Kouhsari, E. et al.	2017	MEA	E-test	12	35	3	2
Mohammad, S. et al.	2017	MEA	Agar dilution	11	21	3	2
Małgorzata, A. et al.	2021	EU	Agar dilution	12	215	0	2
Mikamo, H. et al	2018	JP	Agar dilution	12	108	0	2

3.3. Antimicrobial resistance

Table 4 and Table 5 present the Weighted Pooled Resistance Rate (WPR) of *C. difficile* for three antibiotics, along with the Weighted Pooled Failure Rate of Clinical Cure (WPF) for two of the antibiotic therapies.

Table 4. WPR for three antibiotics.

Antibiotics	Breakpoint (mg/L)	Tested n	Resistance n (%)	WPR	95% CI	Heterogeneity
Vancomycin	≥2	4840	202(4.17)	0.0354	0.0134 - 0.0651	93.90%
Fidaxomicin	≥1	1651	18(1.09)	0.0051	0 - 0.0205	82.70%
Metronidazole	≥2	3705	132(3.56)	0.0371	0.0048 - 0.0904	92.80%
Metronidazole	≥32	252	6(2.38)	0.0370	0 - 0.2677	91.10%

Table 5. WPF of Vancomycin and Fidaxomicin.

Antibiotics	Patients	Cure failure n (%)	WPF	95% CI	Heterogeneity
Vancomycin	3780	832(22.01)	0.2195	0.1645 - 0.2798	94.00%
Fidaxomicin	2003	329(16.43)	0.1757	0.1107 - 0.2516	93.60%

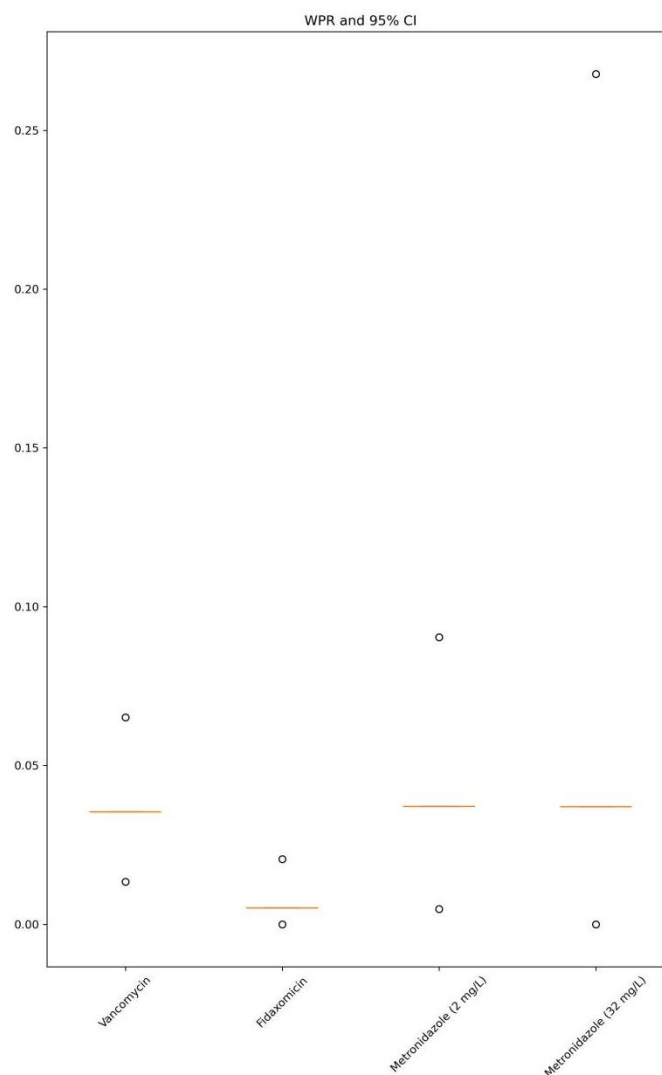


Figure 2. WPR and 95%CI for the three antibiotics.

As figure 2 shows, *C. difficile* weighted pooled resistance rates and 95% CI are presented for vancomycin, fidaxomicin, metronidazole (at the 2mg/L breakpoint), and metronidazole (at the 32mg/L breakpoint). Horizontal lines denote WPR, and dots indicate the range of 95% CI.

3.3.1. Vancomycin. 32 studies were incorporated, involving the examination of 4,861 *C. difficile* isolates. The breakpoint for resistance was 2 mg/L. The WPR was 3.72% (95% CI 1.48% ~ 6.71%) with substantial heterogeneity ($I^2 = 93.7\%$) and no significant publication bias (Peters test, p -value = 0.1863). In the subgroup analysis of the regions from which the literature data came, significant differences were found in this study (p -value < 0.0001) and the highest WPRs were observed in European Union (9.40%, 95% CI 0 ~ 41.80%) followed by North America (8.40%, 95% CI 3.6% ~ 14.6%). Data from 2005 to 2015 (WPR 3.00%, 95% CI 0.54% ~ 6.91%) and from 2016 to 2021 (WPR 4.45%, 95% CI 1.16% ~ 9.21%) was compared, revealed an increase in resistance rate but without significance (p -value = 0.4393).

The WPF was determined by data from 19 studies where 3780 *C. difficile* isolates were tested. The WPF was 21.95% (95% CI 16.45% ~ 27.98%) with substantial heterogeneity ($I^2 = 94.0\%$) and no significant publication bias (Peters test, p -value = 0.6820).

In the regional subgroup analyses, a statistically significant distinction was detected (p -value < 0.0001), with the European Union displaying the highest clinical cure failure rates (42.01%, 95% CI 34.00% ~ 50.23%), followed by the Asia-Pacific region (34.26%, 95% CI 25.57% ~ 43.50%). Data from 2006 to 2015 (WPF 20.66%, 95% CI 15.11% ~ 26.80%) and from 2016 to 2021 (WPF 29.54%, 95% CI 12.45% ~ 49.89%) was compared, revealed an increase in resistance rate but without significance (p -value = 0.3147).

3.3.2. Fidaxomicin. 10 studies selected breakpoint of 1 mg/L where 1651 isolates were tested. The WPR to fidaxomicin was 0.51% (95% CI 0 ~ 2.05%), demonstrating considerable heterogeneity ($I^2 = 82.7\%$) and a lack of significant publication bias (Peters test, p -value = 0.1324). In the regional subgroup analyses, a statistically significant distinction was noted (p -value = 0.0169). North America displayed the highest fidaxomicin resistance rate (3.76%, 95% CI 0.79% ~ 8.35%), while all other regions exhibited rates below 0.01%. Data from 2010 to 2015 (WPR 0.57%, 95% CI 0 ~ 3.68%) and from 2016 to 2018 (WPR 0.33, 95% CI 0 ~ 1.91%) revealed a slight decrease but without significance (p -value = 0.9632).

The WPF was determined by data from 10 studies with 2,003 isolates. The WPF was 17.57% (95% CI 11.07% ~ 25.16%) with substantial heterogeneity ($I^2 = 93.6\%$) and no significant publication bias (Peters test, p -value = 0.2613). In the regional subgroup analyses, a statistically significant disparity was identified (p -value < 0.0001). The European Union recorded the highest rates of treatment failure (42.01%, 95% CI 34.00% ~ 50.23%), followed by the Asia-Pacific region (34.26%, 95% CI 25.57% ~ 43.50%). Data from 2007 to 2012 (WPF 9.33%, 95% CI 6.35% ~ 12.79%) and from 2013 to 2019 (WPF 28.43%, 95%CI 23.18% ~ 33.98%), revealed a significant increase (p -value < 0.0001).

3.3.3. Metronidazole. 32 studies selected breakpoint of 2 mg/L where 3957 isolates were tested. The WPR stood at 3.18% (95% CI 0.56% ~ 7.27%), reflecting notable heterogeneity ($I^2 = 91.6\%$) and a minor degree of publication bias (Peters test, p -value = 0.0405). In the regional subgroup analyses, a statistically significant contrast was established (p -value < 0.0001). The Middle East and Africa region exhibited the highest resistance rate to metronidazole (22.86%, 95% CI 4.41% ~ 48.64%), followed by North America (6.06%, 95% CI 0.12% ~ 17.58%). Data from 2005 to 2015 (WPR 0.68%, 95% CI 0 ~ 2.08%) and from 2016 to 2021 (WPR 5.46%, 95% CI 0.77% ~ 13.10%) revealed a significant increase (p -value = 0.0482).

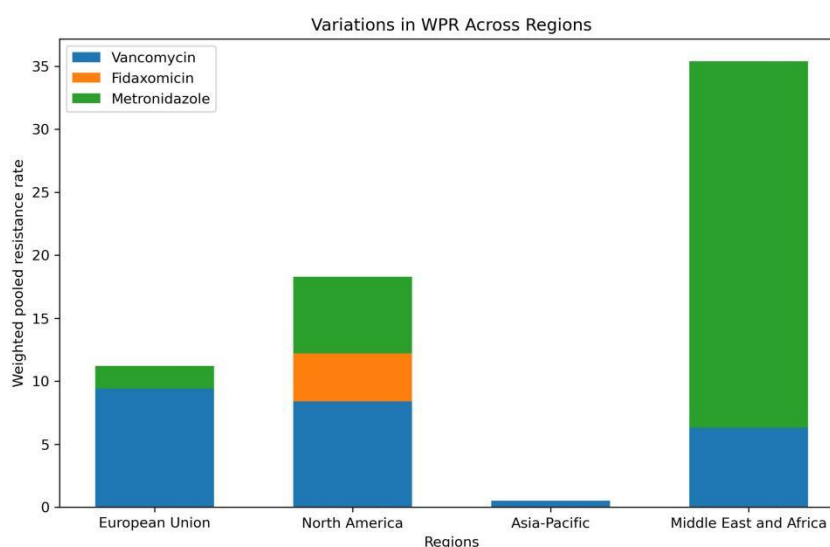


Figure 3. Subgroup analysis about data categorized by regions.

Within four distinct global regions, the respective resistance rates to Vancomycin, Fidaxomicin and Metronidazole are illustrated in the figure 3.

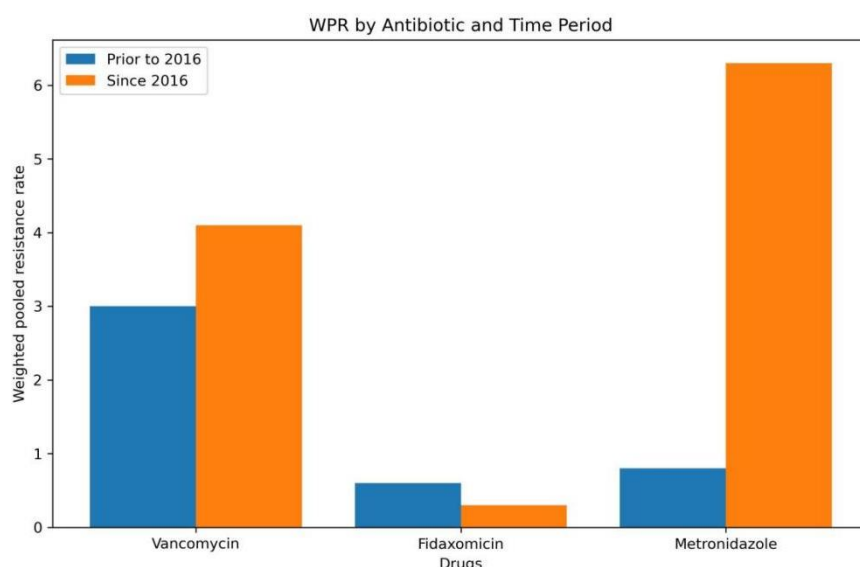


Figure 4. Subgroup analysis about data categorized by years.

Figure 4 compares the disparity in resistance to the same antibiotic between two time periods: prior to 2016 and since 2016.

3.4. Discussion

Previous studies have compared the efficacy and antimicrobial susceptibility of these antibiotics, revealing lower cure rates among patients treated with Fidaxomicin, yet without more comprehensive data on resistance [14]. In contrast, this paper used meta-analysis to separately assess the resistance profile of *C. difficile* against Vancomycin, Fidaxomicin, and Metronidazole in the clinical treatment process of CDI. The data were all sourced from high-quality research to ensure the credibility of the results, and sub-group analysis was conducted to explore differences in resistance rates and clinical cure failure rates among different regions and years.

Metronidazole shows the highest resistance in *C. difficile*, followed by vancomycin, with the lowest resistance seen for fidaxomicin (Fig. 2). Correspondingly, fidaxomicin therapy for CDI is associated with a lower clinical cure failure rate than vancomycin (Table 5), suggesting that *C. difficile* is most sensitive to Fidaxomicin. However, the clinical cure failure rate of Fidaxomicin has significantly increased from 2007 to 2019 (Fig. 4), while its resistance rate has not significantly changed, implying that there may be other factors besides resistance affecting the treatment effect of Fidaxomicin on CDI.

Significant regional variations exist in *C. difficile* resistance to the three antibiotics, notably with high resistance rates observed in North America for all three (Fig. 3). The resistance rate to metronidazole significantly rose from before 2016 to after 2016, highlighting a worsening effectiveness of metronidazole against *C. difficile* (Fig. 4). Furthermore, the combination of Agar dilution and E-test methods for susceptibility testing did not have a statistically significant impact on the results (Supplementary Figure).

This study included 26 literature items (100%) all of which had high-quality methodology with low risk of bias. The analysis of resistance to Vancomycin, Fidaxomicin, and Metronidazole showed no significant publication bias. The limitations of this study include: (i) there were few studies included in the analysis of the clinical failure rate of Metronidazole, which affected its further sub-group analysis

and to some extent the test efficiency of the meta-analysis; (ii) the sensitivity analysis results showed that the unclear severity classification of the disease and the length of follow-up did not affect the initial analysis results, but the inclusion of relevant literature based on disease severity classification may cause potential selection bias.

4. Conclusion

Through the above methods, exploration on the resistance of *Clostridium difficile* to Vancomycin, Fidaxomicin, and Metronidazole in different regions provides important reference value for improving existing clinical antibiotic treatment plans for CDI. Nevertheless, given the constraints of the research methodology, additional confirmation is required through meticulously planned multicenter and extensive-sample clinical trials to establish more efficacious treatment strategies for patients with *Clostridium difficile* infection (CDI).

Authors Contribution

The authors contributed equally to this work, and their names were arranged in alphabetical order.

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The influence of acetaminophen on human health

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Abstract. In pain management, acetaminophen, a widely used analgesic and antipyretic, holds a central position. The metabolic pathways can lead to acetaminophen-induced hepatotoxicity, elucidating from glucuronidation to the cytochrome P450 system. It meticulously details the complexities of acetaminophen metabolism, culminating in the formation of the toxic metabolite, n-acetyl-p-benzoquinone imine (NAPQI). Providing a holistic understanding guiding clinical practice, it explores risk factors, clinical implications, and management strategies. Transitioning to respiratory risks, potential associations between acetaminophen use and asthma, rhinoconjunctivitis, and eczema in adolescents is investigated. The study offers nuanced insights, urging caution in acetaminophen use during adolescence. In addition, the role of drug metabolism in acetaminophen-induced hepatic necrosis is also analyzed. Despite its temporal origins, the study's identification of cytochrome P450 as a catalyst for NAPQI formation remains foundational, contributing mechanistic insights to drug-induced liver injury knowledge. Synthesizing these studies reveals a delicate balance between acetaminophen metabolism and detoxification, crucial in determining its hepatotoxic potential. This nuanced understanding informs clinical practice and beckons researchers to bridge gaps, embarking on further explorations. In essence, this synthesis becomes a compass guiding the scientific community toward a profound comprehension of acetaminophen, epitomizing the standards expected in high-quality, peer-reviewed literature.

Keywords: acetaminophen, human health, hepatotoxic potential.

1. Introduction

Widely known as paracetamol, acetaminophen is a prevalent over-the-counter medication celebrated for its fever-reducing and pain-relieving properties. Over decades, it has been integral to medical practice, providing relief to individuals grappling with diverse ailments [1]. Despite its widespread use and efficacy, the impact of acetaminophen on human health elicits both fascination and concern. This paper embarks on a comprehensive exploration of the multifaceted influence exerted by acetaminophen on human health, elucidating the compelling rationale behind the imperative need to scrutinize this commonplace medication.

The pervasive use of acetaminophen necessitates a comprehensive understanding of its repercussions on human health. From alleviating minor headaches to mitigating severe pain, acetaminophen has firmly established itself as a household remedy. However, its consequences extend beyond symptom relief. Prolonged or excessive usage correlates with deleterious outcomes, notably liver damage. Moreover, a growing body of research suggests potential associations between acetaminophen and other health

conditions, including cardiovascular and respiratory disorders. Given its widespread use, exploring these facets is imperative for fostering well-informed decision-making regarding its consumption.

The study of acetaminophen's impact on human health holds profound significance on several fronts [2, 3]. Firstly, it provides insights into the delicate balance between its benefits and potential risks, equipping healthcare professionals and consumers alike with the necessary tools for judicious choices. Secondly, considering acetaminophen's availability without prescription, promoting public awareness of its health implications is pivotal for guiding responsible usage. Thirdly, in the context of an aging population and the rising prevalence of chronic diseases, understanding the repercussions of long-term Acetaminophen use is crucial, as it often serves as the cornerstone for pain management.

This research aims to achieve specific objectives, shedding light on the influence of acetaminophen on human health. This introduction establishes the framework for a comprehensive exploration of acetaminophen's impact on human health. Through a thorough examination of its effects, benefits, and potential risks, it aims to offer valuable insights that empower individuals to make enlightened choices concerning the use of acetaminophen, while also guiding healthcare professionals in ensuring the well-being of their patients. Subsequent sections of this research will delve deeper into various facets of acetaminophen's influence on human health. The primary goal of this investigation is a comprehensive scrutiny of the effects of Acetaminophen on human health, encompassing an inclusive consideration of both its therapeutic benefits and potential risks. Through this endeavor, it will seek to provide a holistic perspective that empowers individuals and healthcare providers with the insights necessary for informed decision-making regarding its utilization. In pursuit of our research objectives, it will employ a multifaceted approach that includes an exhaustive review of existing literature, meticulous analysis of clinical studies, and a thorough examination of real-world data related to acetaminophen usage. The scope of this study will span various dimensions, covering the pharmacological mechanisms of acetaminophen, its safety profile, recommended dosage guidelines, possible adverse effects, and the dynamic landscape of research concerning acetaminophen.

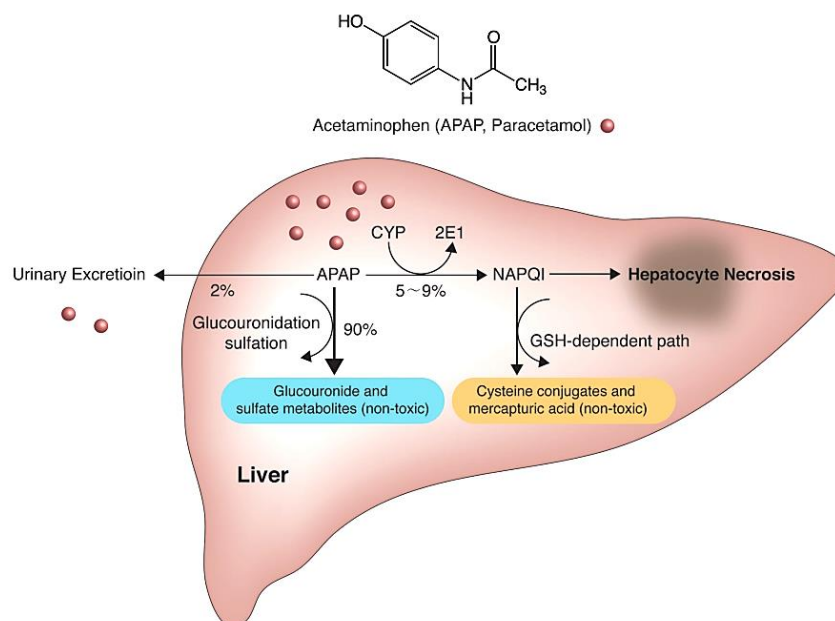


Figure 1. The metabolic pathway of acetaminophen [4].

2. Unraveling acetaminophen-induced hepatotoxicity

Yoon et al.'s seminal work serves as the cornerstone, unravelling intricate mechanisms of acetaminophen-induced hepatotoxicity [4], as shown in Figure 1. This peer-reviewed article meticulously examines the pathophysiological cascade leading to acute liver failure, the foremost consequence of acetaminophen toxicity. It contributes by exhaustively elucidating mechanisms, risk

factors, clinical presentation, diagnosis, and management of hepatotoxicity. From the expansive canvas of glucuronidation and sulfation pathways to the malevolent role of the cytochrome P450 pathway, Yoon et al.'s comprehensive update permeates the scientific discourse. Integration of data-driven evidence substantiates their argument that acetaminophen-induced hepatotoxicity is not only a prevailing concern but, crucially, a preventable and treatable condition with timely recognition and intervention.

Noteworthy is the nuanced exploration of risk factors, extending beyond conventional realms to encompass chronic alcohol use, malnutrition, and specific genetic variations. This expanded understanding augments clinical acumen and underscores the critical importance of tailoring interventions to individual patient profiles. The emphasis on the early administration of n-acetylcysteine as a pivotal therapeutic intervention forms a focal point. Yoon et al.'s meticulous literature review encapsulates the current scientific landscape and significantly contributes to the field by offering actionable insights, shaping the future trajectory of clinical practice in managing acetaminophen-induced hepatotoxicity.

The existing research posits acetaminophen-induced hepatotoxicity as a significant contributor to acute liver failure, with a clarion call for an in-depth understanding of its mechanisms and risk factors. Key conclusions resonate with the preventability and treatability of this condition through early recognition and judicious management, buttressed by the efficacious administration of n-acetylcysteine. The overarching message is clear: in the realm of acetaminophen-induced hepatotoxicity, knowledge is the linchpin that transforms clinical outcomes.

3. Acetaminophen and the risk landscape

Serving as a beacon of inquiry in navigating the intricate association between acetaminophen use and asthma risk, Eneli et al.'s exploration delves into this high-quality, peer-reviewed article [5]. The paper probes potential linkages between acetaminophen use and asthma development, especially in the pediatric domain. Beyond its analgesic and antipyretic roles, acetaminophen emerges as a pharmacological entity with far-reaching implications for respiratory health. Meticulously sifting through diverse studies, Eneli et al. present findings that underscore the nuanced nature of this association. Their exploration of conflicting results, particularly in studies examining pediatric populations, sheds light on the complexities defining the interplay between acetaminophen and respiratory outcomes.

Eneli et al.'s synthesis heralds novel insights into potential risks associated with acetaminophen use, particularly in asthma development. By acknowledging conflicting results in the literature, the authors underscore the imperative for cautious interpretation. Their emphasis on the need for further research serves as a clarion call for a more nuanced understanding of the complex relationship between acetaminophen use and asthma risk, urging healthcare professionals to exercise prudence in prescribing practices. This work encapsulates a thesis positing a potential association between acetaminophen use and asthma risk, unravelling the complex skein of conflicting evidence. The central conclusion underscores the imperative for further research to clarify this relationship and highlights the need for healthcare professionals to exercise caution, particularly in paediatric populations and during pregnancy.

4. Acetaminophen use during pregnancy

In the realm of maternal-fetal pharmacology, Liew et al.'s comprehensive investigation into the association between acetaminophen use during pregnancy and subsequent behavioral problems and hyperkinetic disorders in children stands as an exemplar of scholarly inquiry [6]. This population-based cohort study, conducted in Denmark and involving a substantial cohort of over 64,000 children, delves into uncharted territory. It navigates the intricate landscape of prenatal acetaminophen exposure and its potential repercussions on neurodevelopmental outcomes. Employing detailed questionnaires and rigorous statistical analyses, Liew et al. present a compelling argument for a potential association, unrivalled in its scope and methodological rigor.

The groundbreaking nature of Liew et al.'s work lies in its illumination of the potential behavioral ramifications stemming from prenatal acetaminophen exposure. The study not only identifies a

statistically significant association but ventures further to establish a potential dose-response relationship—a novel insight that transcends the existing literature. Acknowledging the need for further research tempers the findings, reflecting a commitment to scientific prudence and a nuanced understanding of the limitations inherent in epidemiological studies.

Liew et al.'s work asserts a potential association between prenatal acetaminophen exposure and subsequent behavioral problems and hyperkinetic disorders in children. The key conclusions are anchored in robust statistical evidence, suggesting a dose-response relationship. The call for further research echoes the scientific ethos of continuous refinement, underscoring the dynamic nature of knowledge in the intricate realm of maternal-fetal pharmacology.

5. Acetaminophen use and risk in adolescents

In navigating the adolescent terrain, Beasley et al.'s meticulous exploration of the association between acetaminophen uses and the risk of developing asthma, rhinoconjunctivitis, and eczema marks a seminal contribution to the respiratory and allergic discourse [7]. This population-based cohort study, spanning multiple countries and engaging over 300,000 adolescents, employs detailed questionnaires to unravel the potential ramifications of acetaminophen use during this critical developmental phase. The robustness of the study design, meticulous control for confounding factors, and the sheer scale of participation lend credence to the findings, elevating them to the realm of authoritative insights.

The study's revelation of a significant association between acetaminophen uses and an increased risk of asthma, rhinoconjunctivitis, and eczema in adolescents heralds a paradigm shift. By dissecting the association across varying frequencies of acetaminophen use, the authors illuminate a potential dose-response relationship—an incisive insight that augments the granularity of our understanding. Acknowledgment of confounding factors and the call for further research attests to the study's commitment to methodological rigor and scientific integrity. Beasley et al.'s work posits acetaminophen use as a potential harbinger of respiratory and allergic maladies in adolescents. The study concludes with a clarion call for cautious acetaminophen use during this critical developmental phase and underscores the imperative for further research to unravel the intricacies of the observed associations.

6. Unraveling the role of drug metabolism in acetaminophen-induced hepatic necrosis

Embarking on an odyssey through the annals of drug metabolism, Mitchell et al.'s foundational work in 1973 delineates the role of drug metabolism in acetaminophen-induced hepatic necrosis [8]. This experimental study, conducted on mice and rats, pioneers the identification of the toxic metabolite—*N*-acetyl-*p*-benzoquinone imine (NAPQI)—as the malevolent orchestrator of hepatic damage. The study unfolds the biochemical intricacies of NAPQI formation, attributing this process primarily to the catalytic prowess of cytochrome P450. The discerning lens of Mitchell et al. pierces through the veneer of hepatotoxicity, underscoring the pivotal interplay between drug metabolism, toxicity, and detoxification.

Mitchell et al.'s foundational insights not only demystify the mechanistic underpinnings of acetaminophen-induced hepatic necrosis but also illuminate the pivotal role of cytochrome P450 in catalyzing the formation of the toxic metabolite NAPQI. The elucidation of the delicate balance between NAPQI formation and detoxification by glutathione emerges as a linchpin in comprehending the hepatotoxic potential of acetaminophen. By traversing the labyrinth of drug metabolism, the authors lay the groundwork for subsequent research endeavors, offering a foundational understanding that reverberates through decades. Mitchell et al.'s work encapsulates the centrality of drug metabolism in acetaminophen-induced hepatic necrosis, identifying cytochrome P450 as the chief protagonist. The main conclusions delineate the mechanistic intricacies of NAPQI formation and underscore the crucial balance between toxicity and detoxification in the acetaminophen-induced hepatotoxic saga.

7. Synthesis and future trajectories

7.1. Comprehensive landscape

This synthesis transcends the sum of its parts, weaving together disparate threads into a comprehensive

tapestry that encapsulates the multifaceted nature of acetaminophen. From hepatotoxicity to respiratory risks and the intricate web of drug metabolism, the synthesis illuminates a nuanced understanding that befits the standards of high-quality, peer-reviewed literature. The scientific journey mirrored in the existing works embodies a commitment to relentless refinement, transparent acknowledgment of limitations, and an unwavering pursuit of advancing healthcare.

7.2. Knowledge affirmation and gaps illumination

The synthesis affirms existing knowledge while simultaneously casting a discerning eye on the lacunae that beckon further exploration. Yoon et al.'s mechanistic revelations, Eneli et al.'s explorations into respiratory associations, Liew et al.'s maternal-fetal pharmacological inquiry, Beasley et al.'s adolescent odyssey, and Mitchell et al.'s foundational insights into drug metabolism together craft a narrative that illuminates both the potential for harm and healing inherent in acetaminophen.

7.3. Future trajectories

At present, this drug has been widely studied [9, 10]. In this synthesis, the call for further research echoes persistently, underscoring the dynamic nature of scientific inquiry. The recognition of limitations in each study becomes a catalyst for future investigations. And future research trajectories must be guided by meticulous methodologies, expansive cohort sizes, and a commitment to unravelling the intricate nuances that define the pharmacological odyssey of this ubiquitous drug.

8. Conclusion

In navigating the realm of acetaminophen, this synthesis stands as a beacon of scholarly rigor, transcending conventional discourse. The intricacies of hepatotoxicity, respiratory risks, and drug metabolism unfold with precision, guided by the exacting standards of high-quality, peer-reviewed literature. Mechanistic revelations, respiratory associations, maternal-fetal pharmacological odyssey, exploration of adolescence, and a comprehensive narrative is present in this research. Embracing this nuanced understanding equips healthcare professionals, researchers, and the general populace to navigate the acetaminophen landscape with a discerning eye, recognizing its potential for both harm and healing. In essence, this synthesis becomes a compass guiding the scientific community toward a more profound comprehension of acetaminophen, epitomizing the standards expected in high-quality, peer-reviewed literature.

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Unravelling the biochemical mysteries of mycotoxins: Nature's fungal toxins

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Abstract. Understanding the biochemical intricacies of mycotoxin toxicity is essential for effectively addressing these challenges. This comprehensive research delves into the multifaceted world of mycotoxins, beginning with an exploration of their origins, discussing the fungal species responsible for their production, the environmental conditions conducive to their development, and their ecological roles and adaptive significance. The research proceeds to elucidate the biochemical mechanisms underpinning mycotoxin toxicity, describing how these toxins interact with essential biomolecules, disrupt cellular processes, and manifest specific biochemical effects. The research delves into key topics of mycotoxin metabolism and biotransformation in vivo, discussing detoxification processes in the liver, the role of enzymes, and genetic variants that influence susceptibility. The acute and chronic health effects of mycotoxins and the role of biochemical biomarkers in assessing exposure are explored in depth. Synergies and co-exposures are discussed, and the biochemical mechanisms behind interactions with other toxic substances and their implications for risk assessment and public health are discussed. This research underscores the necessity of continuous research in unravelling the biochemical complexities of mycotoxins, emphasizing their impact on agriculture, food safety, and human health, and the urgency of vigilance in addressing these challenges.

Keywords: mycotoxins, biochemical mechanisms, human health.

1. Introduction

In the intricate tapestry of nature's biochemical intricacies, there exists a clandestine world of chemical warfare, where certain fungi unleash potent toxins known as mycotoxins. These enigmatic compounds, produced by various fungal species, are hidden threats that have captivated the attention of scientists, biochemists, and toxicologists. Understanding the intricate biochemistry of mycotoxins and their toxic mechanisms is not only a scientific endeavor but also a crucial step in mitigating their impact on agriculture, food safety, and human health.

Mycotoxins represent a diverse group of secondary metabolites, intricately crafted by fungi belonging to genera such as *Aspergillus*, *Penicillium*, and *Fusarium*. These microorganisms, often found in soil, decaying organic matter, and a myriad of agricultural products, have evolved the production of mycotoxins as part of their biological warfare. This chemical arsenal serves to inhibit the growth of competing microorganisms and protect the fungi from predators, creating a remarkable example of nature's biochemistry at work. The biochemistry of mycotoxins is a multifaceted realm, encompassing their chemical structures, modes of action, and intricate interactions with living organisms. Each

mycotoxin possesses unique chemical properties and exerts its toxic effects through specific biochemical mechanisms. Some mycotoxins, such as aflatoxins, are potent hepatotoxins, while others, like ochratoxins, have nephrotoxic properties. Elucidating the biochemical intricacies of these compounds is pivotal for understanding their impact on human and animal health.

Mycotoxins enter the human and animal food chains through contaminated crops and agricultural products. When ingested, they can interact with biomolecules within the digestive system, setting off a cascade of biochemical events. These toxins may bind to proteins, DNA, and enzymes, disrupting vital cellular processes and potentially leading to acute or chronic health consequences [1]. The biochemistry of mycotoxin toxicity also extends to their metabolism and biotransformation within the body. The liver plays a crucial role in metabolizing mycotoxins, with enzymes facilitating detoxification processes [2]. Genetic variations in these enzymes can influence an individual's susceptibility to mycotoxin-induced toxicity, highlighting the interplay between genetics and biochemistry in toxicology. Moreover, the biochemical pathways of mycotoxin exposure can vary depending on the route of exposure. Whether through ingestion, inhalation, or dermal contact, mycotoxins undergo distinct biochemical transformations within the body, affecting the toxicokinetics and toxicodynamics of these compounds.

This research will uncover the intricate molecular mechanisms that underlie their toxic effects. This exploration will shed light on the biochemical intricacies of nature's fungal toxins, highlighting the importance of ongoing research and vigilance in safeguarding agriculture, food safety, and human health against these hidden threats.

2. Origins of mycotoxins

Mycotoxins are the metabolic by-products of various fungal species, with some of the most well-known producers being *Aspergillus*, *Penicillium*, and *Fusarium*. These fungi are found ubiquitously in the environment, thriving in diverse habitats such as soil, decaying plant matter, and stored grains. Different fungal species are associated with the production of specific mycotoxins, each with its own set of ecological niches and preferred environmental conditions. Understanding the specific fungal sources of mycotoxins is fundamental to addressing contamination in agriculture and food production. The development of mycotoxins is contingent upon a range of environmental factors. Temperature, humidity, and the availability of substrates, such as plant material, influence the growth and toxin production of these fungi. For instance, elevated moisture levels and warm temperatures provide favorable conditions for the proliferation of mycotoxin-producing fungi, making post-harvest storage of crops particularly susceptible to contamination. Therefore, comprehending the environmental conditions that foster mycotoxin development is pivotal in implementing preventive measures to mitigate their presence in agricultural and food supply chains.

Mycotoxins, despite their negative implications for human and animal health, may play pivotal roles in the ecology and survival of the fungi that produce them. These toxins can function as defense mechanisms against other microorganisms and predators, safeguarding the fungus from competition and predation. Mycotoxins may have signalling functions that help fungi adapt to their surroundings. Understanding the ecological roles and adaptive significance of mycotoxins provides insights into the intricate interplay between fungi, their toxins, and the ecosystems they inhabit. This knowledge can contribute to a more holistic approach to managing mycotoxin contamination while respecting the fungi's ecological importance.

Mycotoxins encompass a diverse array of toxic compounds, classified into several major groups based on their chemical structures and biological effects. Notable among these groups are aflatoxins, produced primarily by *Aspergillus* species, and infamous for their carcinogenic properties. Ochratoxins, derived from *Aspergillus* and *Penicillium* species, are known for their nephrotoxic and carcinogenic effects [3]. Trichothecenes, produced by various *Fusarium* species, have garnered attention due to their ability to inhibit protein synthesis and disrupt cellular functions. These are just a few examples, as mycotoxins are categorized into multiple groups, each with its own set of health risks and regulatory limits.

3. Chemical properties and structures of common mycotoxins

Common mycotoxins exhibit distinct chemical structures that contribute to their toxic nature [4]. Aflatoxins, for instance, are characterized by a difuran ring structure, with B1 and B2 forms possessing a bifurcated lactone ring. Ochratoxins feature a chlorinated phenylalanine group, and trichothecenes are characterized by a sesquiterpene core structure. The diversity in chemical structures reflects the varied origins of mycotoxins and their interactions with biological molecules, which ultimately underpin their toxicological effects. Understanding these structures is essential for developing analytical methods and targeted interventions for mycotoxin control.

Mycotoxins owe their toxicity to their ability to interact with crucial biomolecules within living organisms. These interactions are highly specific and can lead to various biochemical disruptions. For instance, aflatoxins have a potent affinity for DNA, causing mutations and potentially leading to cancer [5]. Ochratoxins disrupt cellular metabolism by inhibiting enzymes involved in protein synthesis. Trichothecenes interfere with the ribosome's function, impeding protein production. Mycotoxins can also generate reactive oxygen species, contributing to oxidative stress and cellular damage. These biochemical characteristics of mycotoxins underline their adverse health effects and necessitate a deeper understanding of their mechanisms to develop effective preventive strategies and therapies.

4. Biochemical mechanisms of mycotoxin toxicity

Mycotoxin toxicity primarily hinges on their intricate interactions with vital biomolecules within living organisms. These toxins can form covalent bonds with proteins, altering their structures and functions. Aflatoxins, for instance, attach themselves to DNA, leading to mutations, while ochratoxins interfere with enzymes involved in cellular processes [2]. Moreover, mycotoxins can bind to cellular membranes, impacting their integrity and disrupting signal transduction pathways. Understanding the specifics of these interactions is crucial in deciphering the biochemical basis of mycotoxin-induced toxicity and its repercussions on health.

Mycotoxins disrupt cellular processes through various mechanisms, which ultimately disturb the equilibrium of vital biochemical pathways. Protein synthesis can be impeded, leading to reduced production of essential cellular components. Additionally, mycotoxins can interfere with key metabolic pathways, such as those responsible for energy production or the synthesis of cellular components [6]. These disruptions contribute to a cascade of events that can lead to cell damage, dysfunction, and, in the long term, adverse health effects in exposed organisms.

Different mycotoxins exert unique biochemical effects that correspond to their chemical structures and biological activities. For instance, aflatoxins are potent carcinogens due to their ability to induce mutations by forming adducts with DNA bases [7]. Ochratoxins disrupt cellular metabolism, particularly affecting the kidneys, and have been linked to renal toxicity. Trichothecenes, on the other hand, are characterized by their ability to inhibit protein synthesis, leading to immune system suppression and gastrointestinal issues [8]. By understanding these specific biochemical effects, researchers can better grasp the intricacies of mycotoxin toxicity and develop targeted strategies for prevention and treatment in cases of exposure.

5. Mycotoxin exposure pathways

Exposure to mycotoxins can occur through various routes, each with distinct implications for health [8]. Ingestion is a common pathway, with contaminated food and feed being a primary source. Inhalation is relevant in occupational settings, particularly for individuals working in environments with high levels of airborne mycotoxin-containing dust. Additionally, dermal contact can lead to mycotoxin absorption through the skin. The choice of exposure route can significantly affect the extent of mycotoxin absorption and distribution within the body, as well as the potential health consequences associated with each pathway.

Mycotoxins can undergo biochemical transformations once they enter the body, including processes such as metabolism, distribution, and elimination. The liver plays a central role in detoxifying mycotoxins through enzymatic reactions, which aim to render these toxins less harmful and more easily

excretable [9]. However, some mycotoxins and their metabolites can still exert toxic effects within the body. Understanding these metabolic processes is crucial, as they can influence the duration and intensity of mycotoxin exposure and thus the potential health risks.

The absorption, distribution, metabolism, and elimination of mycotoxins, collectively referred to as toxicokinetic [10], dictate the concentration of these compounds in the body over time. Toxicodynamics, on the other hand, encompasses the biochemical effects mycotoxins have on the body [11], as shown in Figure 1, including their interaction with cellular components and biological pathways. The interplay between toxicokinetics and toxicodynamics is central to assessing mycotoxin-related health risks. It determines how long mycotoxins persist in the body, where they accumulate, and how they exert their adverse effects. Knowledge of these processes is vital in designing effective strategies to reduce mycotoxin exposure and mitigate their harmful impacts on human and animal health [12, 13].

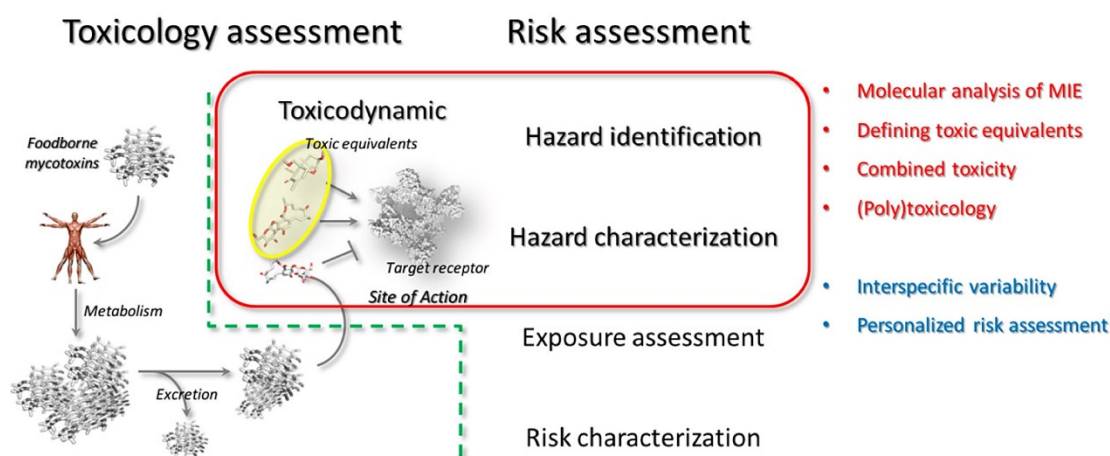


Figure 1. Interconnection between mycotoxins toxicology and risk assessment [11].

6. Metabolism and biotransformation of mycotoxins

The liver, a pivotal organ in the body's detoxification machinery, plays a central role in processing mycotoxins once they enter the bloodstream. In response to mycotoxin exposure, the liver initiates a series of enzymatic reactions aimed at transforming these toxins into less harmful metabolites [12]. This detoxification process involves phase I and phase II reactions, which convert lipophilic mycotoxins into more hydrophilic forms, enhancing their excretion. While the liver's detoxification efforts are generally protective, they can sometimes yield intermediary metabolites that retain toxicity. Understanding these processes is instrumental in comprehending mycotoxin elimination and the potential impact of detoxification on health.

Enzymes play a critical role in mycotoxin metabolism, catalyzing the various reactions involved in detoxification. Cytochrome P450 enzymes, a family of heme-containing proteins, are notably responsible for phase I reactions, which involve oxidation, reduction, and hydrolysis of mycotoxins. Phase II reactions, on the other hand, employ conjugation enzymes like glutathione-S-transferases, sulfotransferases, and UDP-glucuronosyltransferases to attach water-soluble moieties to mycotoxin metabolites, rendering them less toxic and facilitating excretion. The interplay of these enzymes in mycotoxin metabolism is central to the body's defense against mycotoxin-induced harm.

Genetic factors play a significant role in determining an individual's susceptibility to mycotoxin exposure. Polymorphisms in genes encoding detoxification enzymes can influence the efficiency of mycotoxin metabolism, affecting an individual's ability to eliminate these toxins [12]. Genetic variations can lead to differences in mycotoxin clearance rates and the accumulation of toxic metabolites. Additionally, some individuals may possess genetic traits that make them more or less prone to the adverse health effects of mycotoxins. By understanding these genetic variations and their impact on metabolism and susceptibility, researchers can tailor prevention and intervention strategies to specific populations, ultimately enhancing the management of mycotoxin-related health risks.

7. Health effects of mycotoxins

Mycotoxins are notorious for their capacity to induce acute toxicity in individuals exposed to high concentrations of these toxic compounds. Such exposure can lead to a range of symptoms and clinical manifestations, including nausea, vomiting, diarrhea, abdominal pain, and in severe cases, organ failure or death. Aflatoxins, for example, can cause acute liver damage and may lead to acute aflatoxicosis, while the inhalation of mycotoxin-contaminated dust can result in respiratory distress [14]. Understanding the specific symptoms and pathways through which mycotoxins exert acute toxicity is crucial for prompt diagnosis and treatment in cases of poisoning.

In addition to acute toxicity, mycotoxin exposure is associated with a spectrum of chronic health conditions. Prolonged exposure to low levels of mycotoxins, often found in contaminated food and environments, can lead to insidious health effects. These include immune suppression, growth stunting, carcinogenesis, and a range of chronic diseases. Ochratoxins, for instance, are linked to nephrotoxicity and kidney disease. Mycotoxins can also contribute to immunosuppression, leaving individuals more susceptible to infections. Understanding the biochemical mechanisms that underlie these chronic health conditions is paramount for assessing long-term risks and implementing preventive measures.

Biochemical biomarkers play a crucial role in assessing mycotoxin exposure and associated health effects. By measuring specific molecules or indicators in biological samples, such as blood or urine, researchers and clinicians can identify the extent of exposure and assess the potential risks. Biomarkers can reveal the presence of mycotoxin metabolites, indicate the level of toxin exposure, and provide insights into the biochemical processes triggered by mycotoxins within the body. These biomarkers not only aid in diagnosis but also facilitate the monitoring of mycotoxin-related health conditions, enabling timely interventions and targeted strategies for individuals at risk.

8. Synergistic effects and co-exposure

Mycotoxin exposure often occurs alongside exposure to other toxic substances, resulting in intricate interactions. These interactions can amplify the adverse effects of mycotoxins and other toxins, potentially leading to more severe health consequences. Understanding how mycotoxins interact with these co-exposures is crucial for a comprehensive evaluation of health risks.

Synergistic toxicity involves the biochemical mechanisms by which mycotoxins and other toxic substances intensify each other's harmful effects. This may result from cumulative damage to cellular components or interference with essential biochemical pathways. The understanding of these mechanisms aids in discerning how co-exposure impacts health and can inform the development of more effective risk assessments.

The recognition of synergistic effects and co-exposure has profound implications for risk assessment and public health policy. It necessitates a broader perspective on chemical exposures and emphasizes the need for comprehensive strategies that account for the combined impact of multiple toxic substances. Such insights are instrumental in shaping regulations and interventions aimed at safeguarding public health and reducing the risks associated with mycotoxin and co-exposure.

9. Analytical techniques for studying mycotoxins' biochemistry

Accurate and sensitive methods for detecting and quantifying mycotoxins in biological samples are pivotal for understanding the extent of exposure and assessing health risks. Various analytical techniques, such as liquid chromatography-mass spectrometry (LC-MS) and enzyme-linked immunosorbent assays (ELISA), are employed for this purpose. These methods play a critical role in research, regulation, and public health, enabling the precise measurement of mycotoxin concentrations.

The field of mycotoxin analysis continually evolves with the emergence of advanced analytical tools and technologies. Innovative approaches, including high-resolution mass spectrometry and genomics-based methods, offer enhanced accuracy and efficiency in mycotoxin detection and quantification. Staying abreast of these technological advances is vital for maintaining the highest standards in mycotoxin research and regulation.

Accurate mycotoxin analysis is a cornerstone of both research and regulation. In research, it provides the basis for understanding exposure patterns, biochemical mechanisms, and health effects. In regulation, it informs safety standards and quality control measures for food and feed production. The reliability of analytical techniques directly influences the assessment and management of mycotoxin-related health risks, making precision and quality in analysis imperative.

10. Conclusion

Current research in mycotoxin biochemistry is characterized by a dynamic and evolving landscape. Scientists worldwide are actively investigating the latest developments in mycotoxin detection, exposure assessment, and health effects. This ongoing research expands our knowledge of mycotoxins and contributes to the development of more effective strategies for prevention and intervention. Despite significant progress, emerging issues and challenges continue to shape the field of mycotoxin biochemistry. Factors like climate change, altered agricultural practices, and evolving fungal strains pose new challenges in managing mycotoxin contamination. Additionally, the interplay between mycotoxins and human health remains a subject of constant scrutiny, underscoring the need for vigilance in addressing these challenges.

There are some potential directions for improving mycotoxin safety and mitigation. The future of mycotoxin safety and mitigation hinges on the development of innovative strategies and technologies. Potential directions include precision agriculture to reduce mycotoxin contamination at the source, advanced mycotoxin control measures, and the use of predictive modelling to anticipate mycotoxin outbreaks. These approaches hold the promise of enhancing food and feed safety and reducing the health risks associated with mycotoxin exposure. Throughout this exploration of mycotoxins, we have delved into their origins, classification, modes of action, exposure pathways, metabolism, health effects, synergistic interactions, analytical techniques, and the current state of research. We have unraveled the biochemical intricacies that underlie mycotoxin toxicity, from acute effects to chronic health conditions, considering the role of biochemical biomarkers and co-exposure with other toxic substances.

Mycotoxins continue to pose a formidable threat to agriculture, food safety, and human health. The knowledge we gain from unravelling their biochemical mysteries directly informs our ability to assess risks, develop preventive measures, and safeguard the well-being of both humans and animals. The agricultural sector relies on advancements in mycotoxin research to secure food supplies and prevent economic losses. As a cornerstone of public health, understanding mycotoxins' biochemical complexities is paramount in ensuring the safety of the global food chain. In conclusion, our exploration of mycotoxins underscores the imperative of continued research and unwavering vigilance in addressing the biochemical complexities of these fungal toxins. The ever-evolving challenges presented by mycotoxins demand ongoing efforts to refine our understanding, detection methods, and risk management strategies. This necessitates a multidisciplinary approach, bringing together scientists, regulators, and industries to collaboratively mitigate the adverse effects of mycotoxin exposure. As we look ahead, the biochemical mysteries of mycotoxins will continue to unravel, paving the way for a safer, more secure future in agriculture, food safety, and public health.

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DNA methylation-linked diseases and analytical methods

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Abstract. As people investigate deeply into the genetic level of the causes/mechanisms behind serious diseases, scientists found out that epigenetics can explain some of the causes of serious diseases such as cancer. The study of epigenetics focuses on the differential gene expression of a DNA sequence that does not involve any changes to the original sequence. Proteins that interact with DNA and DNA letters themselves have the chemical ability to change how much a gene is switched on or off. Certain epigenetic changes can be passed from parent cells to the daughter cells during cell division. There are various mechanisms of epigenetics, and DNA methylation was one of the earliest epigenetic mechanisms to be discovered. DNA methylation controls gene expression by either attracting proteins that play a role in the regulation of genes or by preventing transcription factors from binding to DNA sequence. This overview introduces several research studies of serious diseases: breast cancer, autism spectrum disorder, and obesity that are caused by DNA methylation and some analytical methods: bisulfite conversion-based assays and enzymatic methyl sequencing which are used to investigate DNA methylation.

Keywords: Epigenetics, DNA methylation, Cancer, Bisulfite sequencing

1. Introduction

The study of heritable differences in gene expression or function that are not caused by changes in the sequence of the deoxyribonucleic acid (DNA) is known as Epigenetics. Not all types of cells will have the same gene expression at once, even though an organism's cells nearly all share the same genetic materials. Epigenetic mechanisms, in a larger sense, regulate the varied patterns of gene expression in many different kinds of tissues and cell types in multicellular creatures. DNA methylation was first identified in mammals when DNA was first recognized as the genetic material. Using paper chromatography, Rollin Hotchkiss made the initial discovery of modified cytosine in a calf thymus sample in 1948 [1].

DNA methyltransferases (DNMTs) covalently add a methyl group to the C-5 position of the DNA cytosine ring, resulting in DNA methylation, an epigenetic alteration. In somatic cells, more than 98% of DNA methylation occurs in a CpG dinucleotide environment, nevertheless in embryonic stem cells (ESCs), up to 25% of total DNA methylation occurs in non-CpG settings. Most DNA methylation is essential for several important activities, including X-chromosome inactivation and genomic imprinting. DNA methylation can also be improper and lead to illnesses like cancer [2]. The methylation of DNA is regulated by a family of DNMTs, including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. The majority of CG dinucleotides in mammals are methylated on cytosine residues, although CG

dinucleotides found in promoters are typically shielded from methylation. Global DNA hypomethylation and locus-specific CpG island (CGI) hypermethylation are epigenetic indicators of malignancy. All of the cancer specimens that have been analyzed so far exhibit overall decreases in DNA methylation. The primary underlying cause of hypomethylation is a lack of methylation at repetitive elements that are typically strongly methylated, such as satellites and retrotransposons, which results in genomic unpredictability and oncogene stimulation. Typically, suppressive tumor genes' promoter CGIs experience locus-specific hypermethylation, which causes transmissible transcriptional silence [2].

One of the most significant inventions that helps scientists identify the patterns of methylation is the development of DNA sequencing. DNA sequencing implies to a common analytical procedure for figuring out the specific arrangement of nucleotides, also called bases, in a DNA molecule. The biological information that living organisms use for development and function is stored in the DNA sequence. Identifying the roles of genes and other components of the genome has become achievable as a consequence to the advancement in the availability of genetic information in our genomes made possible by DNA sequencing [3].

This review described three different serious diseases whose causes have been studied to be related to DNA methylation. Moreover, it introduced two different sequencing techniques utilized to analyze DNA information for identifying methylation patterns. By utilizing these advanced DNA sequencing techniques helps scientists to investigate the genetic-level causations of serious diseases, which provides potential effective treatments for these diseases.

2. DNA methylation-linked diseases

Mammalian embryogenesis and optimal adult organism operation depend on appropriately created and retained DNA methylation patterns. DNA methylation is a powerful method for suppressing gene expression and maintaining genomic integrity in the face of this abundance of repetitive DNA. Dnmt1 and Dnmt3b are required for the development of embryos, and mice lacking Dnmt3a passed away shortly after birth, according to research studies of mouse knockout. Additionally, the somatic cells lose the ability to control their proliferation when their DNA methylation patterns revert to normal. The prevalence of human diseases emphasizes the significance of DNA methylation [4]. The following section presents the research findings of three different diseases.

2.1. Breast cancer

The most common hormone-dependent malignancy that causes a high rate of mortality as well as morbidity is carcinoma of the breast. This frequent cancer begins as a hormone-responsive (HR), non-metastatic cancer throughout the intricate, multi-step process of tumor promotion, then gradually transforms into a highly metastatic hormone-insensitive (HI) version that lacks the functioning estrogen receptor. Several malignancies, including breast cancer, have been linked to the progression of urokinase (uPA), a member of the serine protease family. In this study, scientists have investigated the relationship between uPA expression and hormone sensitivity in HR normal mammary epithelial cells, MCF-7, and T-47D breast cancer cell lines [5].

To performing experiments, researchers utilized human mammary epithelial cell line (HMEC). In order to attain the final results, researchers utilized northern blot analysis, nuclear run-off assay, Matrigel invasion assay, and southern blot analysis [5].

DNA from the genome was extracted from HR and HI mammary epithelial cells with the aim of investigating the significance of the methylation of DNA in the variation in the regulation of control of uPA gene activity throughout cancer metamorphosis and tumor development. Total cellular DNA was digested using either Pst 1, a non-methylation-sensitive endonuclease, only (Fig. 2; lanes 1, 4, 7, and 10), or Pst 1 combined with either Hpa11 or Hha1, a methylation-sensitive endonuclease (lanes 2, 5, 8, and 11). Southern blotting was used to determine whether the Hpa11 and Hha1 sites were methylated. A 778 bp Sma I and Avr II promoter region within the uPA gene was used to probe all blots. In HR HMEC, MCF-7, and T-47D cells, methylating the uPA gene's CpG island prevented DNA from being digested by Hpa11 and Hha1, resulting in patterns of restriction that were the same as those of Pst 1

digestion alone (Fig. 1; lanes 1–9). Hpa11 and Hha1 completely digested the genomic DNA to produce many lower bands as opposed to Pst1 digestion alone in H1 MDA-231 cells when the CpG island of the uPA gene was methylation or hypomethylated (Fig. 1; lanes 10–12) [5].

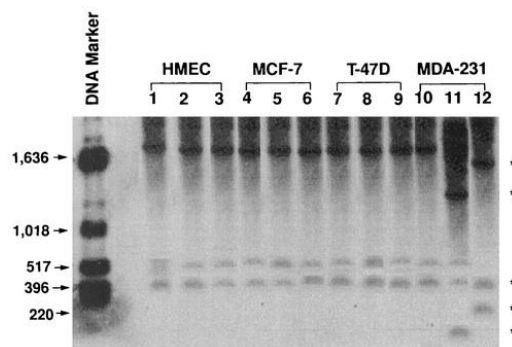


Figure 1. Methylation status of key sites on the human uPA gene's CpG island. Genomic DNA isolated from HR (HMEC, MCF-7, T-47D) and H1 (MDA-231) mammary epithelial cells [5].

Their results provide compelling evidence for an epigenetic pathway involving the methylation of DNA that regulates the transcription-dependent control of uPA gene expression and allows for the on and off regulation of this vital protease at various phases of breast cancer.

2.2. Autism spectrum disorder

A series of neurodevelopmental diseases known as autism spectrum disorder (ASD) are characterized by impaired social interaction and communication, as well as more frequent repetitive activities and narrowed interests. All races, all ethnic groups, and all socioeconomic categories are affected by the condition, which affects males more commonly than females (by a ratio of 4.5 to 1). Even if the majority of ASD cases are not inherited, the substantial consistency of ASD among identical twins points to a significant genetic link as well as complicated transmission. The etiologic involvement of genetic flaws in ASD has also been convincingly demonstrated by genomic findings during the previous two decades [6]. In this research, scientists employed nuclease-deactivated Cas9 (dCas9) coupled with DNA methyltransferase catalytic domains and five locus-targeting sgRNAs to accomplish locus-specific methylation at the location of the transcription start site (TSS) of *Mecp2* in Neuro-2a cells and mice. They investigated the connection between ASD symptoms and locus-specific DNA methylation [7].

To get the most accurate results, the researcher utilized plenty of different techniques during the experiments, including T7EN1 cleavage assay and sequencing, immunofluorescent staining, behavioral assessments pertaining to motor skills, anxiety, and depression, and reduced representation bisulfite sequencing [7].

After researchers methylated the promoter of *Mecp2*, they discovered that male mouse from the experimental methylation collections had considerably less *Mecp2* transcription in the hippocampus and parietal cortex when compared to their control group. Additionally, in contrast to masculine mouse serving as controls, the MeCP2 levels in tissue sections were much reduced as shown by a Western blot analysis, which was supported by a weak immunofluorescence labeling of the MeCP2 protein (Fig. 2D, E) [7].

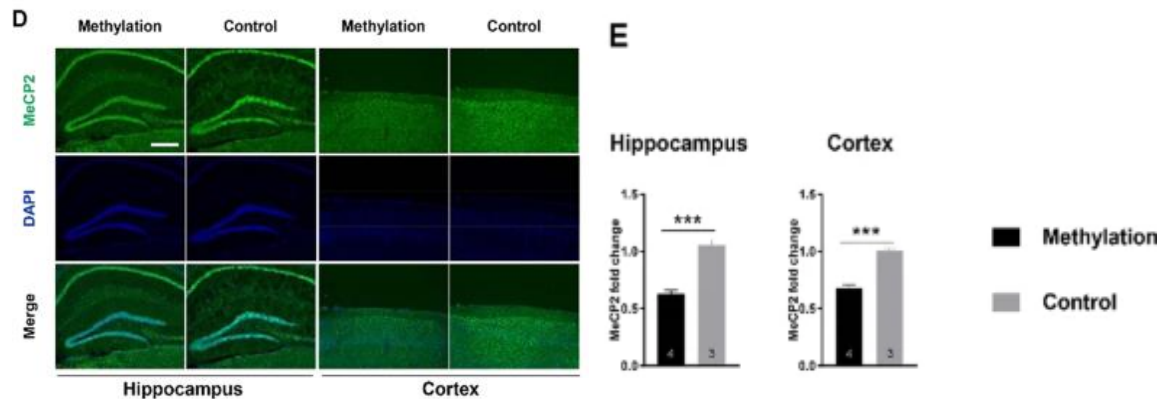


Figure 2. Reduced expression of Mecp2's by targeted DNA methylation [7].

Then, researchers examined whether changing the DNA methylation at the Mecp2 promoter would result in altered mouse behavior. The interpersonal communication test consisted of three separate chambers. When confronted with unknown mouse, mouse in the untreated group preferred to investigate them instead of known mice, while mice in the methylation sample showed no preference between known and unknown mice (Fig 3B). Additionally, we discovered that mouse from the methylation collections spent noticeably more time grooming themselves (Fig. 3C), which indicates an increase in repetitive behavior [7].

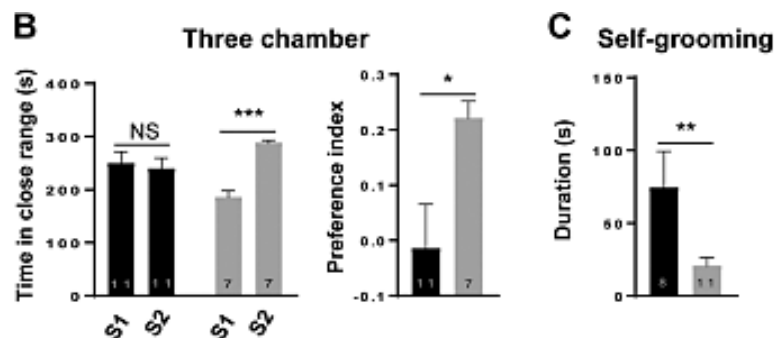


Figure 3. Mecp2's targeted DNA methylation in mice resulted in characteristics similar to ASD [7].

By adopting an enhanced dCas9-based methylation targeting approach to methylate Mecp2 promoter at a specific location, scientists were able to illustrate the crucial role that methylation plays in the pathophysiology of ASD. Mecp2 gene expression was decreased as a result of methylating the Mecp2 promoter. Additionally, the data revealed that methylation group animals were less interested in social novelty or conspecific recognition than control mice.

2.3. Obesity

Over the past few years, childhood obesity prevalence has grown significantly and quickly, turning it into a global public health emergency. In order to determine the methylation biomarkers in obesity, researchers investigated into genome-wide methylation of the DNA in this study. In order to identify the distinctively methylated genes, they used extensive DNA methylation characterization of promoters of genes and CpG islands in obese Chinese preschoolers. This was followed by methylated DNA immunoprecipitation and hybridization to the NimbleGen Human DNA Methylation 385K Promoter Plus CpG Island Microarray [8]. In the study, 748 children from kindergartens in Beijing, including 32 obese preschoolers aged 3 to 6 and an equal number of lean (normal weight) youngsters who were age- and gender-matched, were included. Scientists utilized immunoprecipitation, DNA labeling and array hybridization, and sequencing of bisulfite-modified DNA to obtain their final results [8].

In comparison to lean children, 251 promoters were demethylated in the peripheral blood cells of obese kids, while 141 were hypermethylated. These promoters were connected to a total of 232 and 132 genes, respectively. In addition, when comparing obese children to control group youngsters, 575 CpG islands were demethylated and 277 were hypermethylated (Fig. 4). Further investigation revealed that their chromosomal distribution was unbalanced, particularly on chromosomes 3, 16, 17 and 19, which contained more promoters and demethylated CpG islands. Additionally, it was discovered that chromosome X had more CpG islands and promoters with differential methylation than Y chromosome [8].

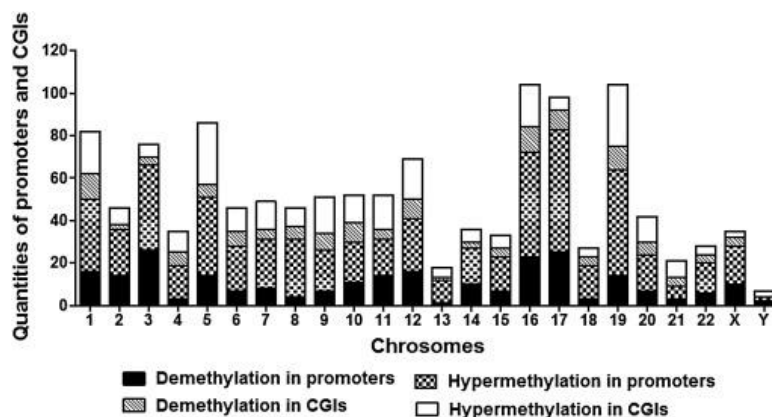


Figure 4. Distribution of CpG islands (CGIs) and promoters with varied levels of methylation in obese children's chromosomes [8].

Furthermore, of the 80 most significant promoters and CGIs with DNA methylation distinctions among control and obese children, almost fifty percent have been studied previously, and the majority of these are associated with the etiology of malignancies that are connected to multiple body parts, including glioblastoma, musculoaponeurotic fibrosarcoma, lung carcinoma, leukemia, and malignant melanoma [8].

3. DNA methylation-linked analytical methods

DNA sequencing played a very crucial role in mapping out the human genome, and it's still a significant tool for both fundamental and applied scientific applications today. As an example, it has provided an essential instrument for determining the numerous numbers of nucleotide variations connected to specific genetic diseases, which may aid in improving understanding of these conditions and therapy. Following the discovery of the mechanisms underlying methylation, scientists came upon sequencing analysis that was specifically utilized to identify the methylation patterns of CpG, CHH, and CHG sites throughout the human genome [9]. The following section introduces two different sequencing techniques that are very helpful for researchers to conduct their studies about methylation.

3.1. Bisulfite conversion-based assays

The gold-standard technique: DNA bisulfite conversion followed by sequencing is used to quantify patterns of methylation at the base-pair level. Both on microarrays and by NextGen sequencing, the first step in assessing DNA methylation can be bisulfite conversion-based methods [10]. Frommer et al. first introduced the bisulfite treatment in 1992 [11].

Researchers utilize the bisulfite sequencing to investigate 5-methylcytosine (5mC) in the single strands sequence. Unmethylated cytosines are converted to uracil by the bisulfite process, causing U to be read as T following PCR amplification and sequencing. Methylated cytosines are unaffected by this change and remain C in the sequence (Fig. 5). To determine the status of DNA methylation, PCR amplicons created following DNA from the genome that has undergone bisulfite conversion can be matched to microarrays containing methylation-specific oligonucleotides (MSOs), which are composed

of 19–23 nucleotides. The fluorescence intensity determines the extent of the variations in hybridization, might provide information on the methylation state of a specific locus since MSO probes could distinguish between methylated and unmethylated cytosines within a given CG-rich sequence. Additional research using MSO allowed for the classification of different human tumor types according to methylation patterns. Studying colorectal cancer can be achieved by a modified version of MSO technology that shows the methylation status of the MGMT gene's promoter region [10].

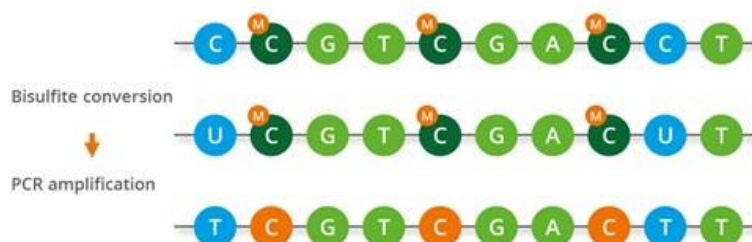


Figure 5. The mechanisms of Bisulfite conversion-based assays [12].

3.2. Enzymatic methyl sequencing

At single-base resolution, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are found using bisulfite sequencing. However, DNA is harmed by bisulfite treatment, leading to DNA loss, fragmentation, and inaccurate sequencing results. Enzymatic methyl-seq (EM-seq), an fully enzyme-based technique for assessing the methylation state of cytosine, was created to address these issues [13].

Three enzymes and a pair of reactions are needed for the enzyme-mediated identification of 5mC and 5hmC. T4-BGT and Tet methylcytosine dioxygenase 2 (TET2) are employed to shield 5mC and 5hmC from APOBEC3A's ensuing deamination. TET2 has the ability to catalyze the oxidation of 5mC to 5hmC, then 5-formylcytosine (5fC) to 5-carboxycytosine (5caC), resulting in simultaneous production of CO₂ and succinic acid (Fig. 6A). TET2-formed and genome-wide 5hmC are both glucosylated to 5-(β-glucosyloxymethyl) cytosine (5gmC) by T4-BGT (Fig. 6B). Then, cytosines are deaminated by APOBEC3A (Fig. 6C) [13].

With fewer sequencing duplicates than bisulfite sequencing, EM-seq produced more usable reads, which increased the effective genome coverage.

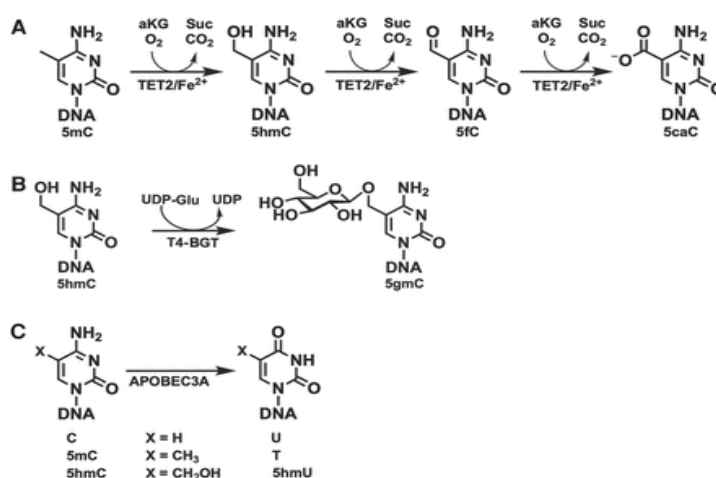


Figure 6. Enzymes that are utilized to detect 5mC and 5hmC [13].

4. Conclusion

Based on the technologies that have been developed to study genetics and genomics, scientists have been studying the reasons behind serious diseases at the genetic level for many years. The DNA/histone

modifications of epigenetics have also become one of the most popular research fields in recent years. This overview introduced the background, methods, and results of three diseases with different etiologies related to DNA methylation: breast cancer, autism spectrum disorder, and obesity and two different sequencing methods used to identify DNA methylation patterns: bisulfite conversion-based assays and enzymatic methyl sequencing. Due to the lack of objective data, this overview does not include the data from patients' clinical trials on autism spectrum disorder that can support the conclusion. With the advanced discoveries and developments of technology, it becomes possible for researchers to study more and more diseases to discover the fundamental causes behind them in the future, and some of their causes might be related to epigenetics. With the knowledge of the causes of diseases, scientists can invent better therapies for treating serious diseases.

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Intranasal alpha-adrenergic receptor agonists and corticosteroids as medical treatments for nasal congestion

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Abstract. Nasal congestion has been a longstanding issue which exerts burdens on both individual patients and society as a whole. It arises from various underlying illnesses like nasal polyposis, rhinosinusitis, allergic rhinitis and rhinitis medicamentosa, but the fundamental mechanism of induced nasal congestion is nasal mucosa's swelling as a result of inflammation in all of these cases. Among all the therapies currently available, intranasal sprays of alpha-adrenergic receptor agonists or corticosteroids appeared to be prominent options. After reviewing research on treatments extensively, limited studies focused on the comparison of α -adrenergic agonists and corticosteroids. This research explores the mechanisms, efficacy, and safety of these two medical approaches. Intranasal alpha-adrenergic receptor agonist sprays target the smooth muscles for vasoconstriction, acting rapidly to give way to airflow. They are effective as a treatment for short-term nasal congestion, otherwise, continued use may lead to rhinitis medicamentosa. Corticosteroid sprays, on the other hand, aim to reduce the severity of nasal inflammation via suppression of pro-inflammatory molecules. They are a promising treatment barely with concerns about the occurrence of side effects, authenticated by their excellent pharmaceutical performance over years.

Keywords: Nasal congestion, Alpha-adrenergic Receptor agonists, Corticosteroids, Rhinitis medicamentosa.

1. Introduction

Nasal congestion is generally defined as insufficient airflow to the nasal cavity sensed by the patient. Scientists define irreversible blockage of the nasal airway due to permanent structural changes as nasal obstruction. Still, the discrepancy is not considered in most research, so this research will not distinguish between these two terms. The most common symptoms are leaking mucus that results in an added effort to clean up due to rhinorrhoea, undermined quality of sleep which induces daytime sleepiness because of the stuffy nose at night, and mouth breathing leading to craniofacial growth disorders [1].

Nasal congestion inflicts sufferers and society in multiple ways, including direct costs involved in treating. Indirect costs arising from disturbed working and studying activities are also a significant component of the burden brought by nasal congestion [2]. For example, the constant sense of fullness in the nose brings substantial frustration. The stuffy nose inhibits patients' ability to speak with clarity during socialisation. Disrupted sleep causes issues with combating fatigue and struggling to concentrate. In cases of severe congestion, decreased enjoyment of life also stems from losing smell. The consequent

economic burden is never negligible. A 2020 study on chronic rhinosinusitis with nasal polyps (CRSwNP), whose main symptom is nasal congestion, found that the additional costs associated with CRSwNP per patient in Europe were approximately €1501, and the indirect costs associated with decreased productivity were €5659 per patient in a year [2].

There are already some feasible medical treatments available. Antihistamines reduce symptoms like sneezing and itching [3]. Intranasal corticosteroids greatly counter nasal mucosa inflammation. Leukotriene receptor antagonists (LTRAs), although not as effective as corticosteroids, mitigate allergic rhinitis. Numerous biologics, such as omalizumab, an anti-IgE antibody, and dupilumab, an antibody that binds to IL-4R α , reduce inflammation by decreasing the amounts of their respective targets [4]. Allergen immunotherapy builds tolerance to allergens in allergic rhinitis [3]. Decongestants including alpha-adrenergic receptor agonists are short-term resorts for nasal congestion [5].

This research will comprehensively contrast the two main types of medical treatment for nasal congestion, alpha-adrenergic agonists and corticosteroids, in terms of mechanism of action, efficacy and possible side effects based on previous research and reviews, as a gap is found in focusing on comparing these two therapies, despite there are plenty of essays concentrating on only one of these drugs or giving a more thorough but less specific overview about nasal congestion. This research will facilitate the decision of use between alpha-adrenergic agonists and corticosteroids and provide prospective directions in future nasal congestion research.

2. The causes of nasal congestion

2.1. Allergic rhinitis

Immunoglobulin E (IgE), an antibody secreted by B cells in response to allergen exposure, binds to the surface of basophils and mast cells. During a subsequent invasion, the allergen attaches itself to these cells' IgE antibodies, causing changes in gene transcription that result in the release of several chemokines and cytokines. These pro-inflammatory molecules include leukotrienes (IL) and histamine. The lining of the nasal cavity is infiltrated by inflammatory cells, such as mast cells, eosinophils, neutrophils, basophils, macrophages, and T lymphocytes [5]. The majority of those T cells are T helper 2 (Th2) cells, which release IL-3, 4, 5, and 13, which further encourage plasma cells to produce IgE. Arterioles dilate, vascular permeability increases, and rhinorrhoea and mucous secretion occur as a result [3], obstructing the nasal cavity.

2.2. Rhinosinusitis

Since rhinosinusitis is initially brought on by nasal inflammation, the term rhinosinusitis is now preferred over sinusitis. Rhinosinusitis is classified as acute or chronic based on whether it lasts less than 12 weeks or longer [5]. There are many similarities between the symptoms of allergic rhinitis (AR) and rhinosinusitis, including nasal discharge and nasal congestion. However, rhinosinusitis patients are more likely to experience facial pressure or pain, as well as anosmia or hyposmia (loss of scent or diminished sensitivity) [5].

2.3. Nasal polyposis

The internal mucosa has a thin layer of loose areolar connective tissue called the lamina propria. Due to the invasion of pathogens, a Th-2 response occurs, resulting in the secretion of Th2 cytokines and subsequently the induced accumulation of IgE and eosinophils. Nasal polyps are eventually formed when the sub-epithelial stroma is remodelled. It is again linked to inflammatory conditions that cause nasal congestion, such as chronic rhinosinusitis, asthma, and aspirin-exacerbated respiratory disease (AERD) [6].

2.4. Rhinitis medicamentosa

'Rhinitis medicamentosa' (RM) or 'rebound congestion' belongs to drug-induced rhinitis, and is caused by overusing topical nasal decongestants (usually more than 7 days) [5]. There are various intranasal

decongestants, including ephedrine, phenylephrine, naphazoline, oxymetazoline, xylometazoline and so forth [7]. It accounts for one percent to nine percent of visits to allergy or otolaryngology clinics [8]. There has not been a definitive explanation of its pathophysiology, however, several reasonable hypotheses have been developed. There is a hypothesis that chronic vasoconstriction results in ischemia of the nasal mucosa and nasal oedema. The second hypothesis describes that congestion and reactive hyperaemia occur when the constrictor mechanisms are exploited, and sensitivity to decongestant molecules decreases. Higher doses are required for consistent use due to enhanced tolerance. Another theory is that greater vascular permeability and oedema stem from alterations in the vasomotor tone [7]. After being informed about rhinitis medicamentosa, the continued use of intranasal decongestants must be stopped [7].

3. Mechanisms of action

3.1. *Alpha-adrenergic agonists*

G-protein coupled receptors (GPCR) for alpha-1 and -2 adrenergic receptors are found at the postsynaptic membrane of vascular smooth muscles [9]. Alpha-1 receptors are Gq type receptors. Following these receptors' activation, phospholipase C (PLC) is activated. PLC catalyses the production of IP₃ and DAG which have roles in signalling. IP₃ induces the endoplasmic reticulum to release calcium ions upon entering the cytoplasm, and the resultant elevated intracellular calcium concentration causes smooth muscle to contract [10]. The stimulation of alpha-2 receptors has also been proven to relieve nasal congestion via contraction of nasal mucosa due to its effects on the vasoconstriction of capacitance vessels in different species [11].

3.2. *Corticosteroids*

The adrenal cortex produces the hormones known as corticosteroids (CSs). Glucocorticoids (GCs) and mineralocorticoids are included in CS. GCs mainly exhibit anti-inflammatory and immunosuppressive properties. In order to prevent inflammation or the influx of inflammatory cells into the airway, GCs have an effect on the cells that line the airway, controlling gene transcription through glucocorticoid receptors (GRs), a subclass of nuclear receptor. Pro-inflammatory cytokines like TNF- α , IL-1 β , and IL-6, as well as their receptors, have their expression inhibited [12]. Hence the reduced level of inflammation mitigates nasal congestion.

3.3. *Comparison*

Alpha-adrenergic receptor agonist sprays can cause contraction of smooth muscles after binding to their receptors to lower the extent of nasal congestion rapidly. They affect the eventual manifestation of the congestion-causing disease. Meanwhile, the prolonged use of intranasal corticosteroids allows them to present anti-inflammatory effects which ultimately suppress nasal cavity inflammation. They alter the fundamental mechanism that causes nasal congestion.

4. Efficacy

Imidazolines oxymetazoline and xylometazoline are major α -adrenergic receptor agonists as nasal decongestants. However, they have slight differences in mode of action and therefore efficacy due to discrepancies in affinity and potency at the α -adrenergic receptor subtypes [13]. 0.1% xylometazoline has a great decongestant effect for up to 10 hours in contrast to placebo sprays. It is an effective resort for treating nasal congestion and does not present rebound congestion within 10 days [14], as shown in Figure 1. The efficacy of oxymetazoline (0.05%) spray for the first time was examined to relieve nasal congestion for a maximum of 12 hours in one dosage [15].

INCS administration is fast and simple. CSs are superior to most agents and are a first-line therapy of AR [5, 16]. One medication that has been shown to be useful in treating nasal congestion brought on by seasonal allergic rhinitis is mometasone furoate (MF). The symptoms can be relieved with MF nasal spray (MFNS) administered once a day and further improved with continuous use for over 14 days.

With a duration of 15 days of MFNS administration, 18% more patients reported a >30% improvement in nasal congestion than the placebo group, and there are 6% more patients who received MFNS responded with a >50% relief than the control group [17].

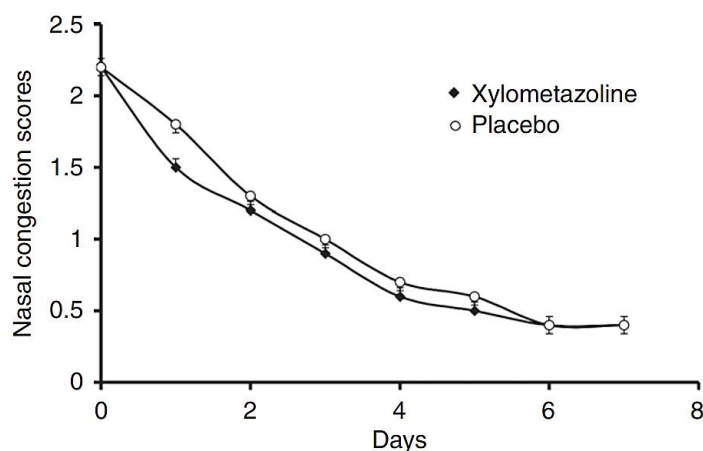


Figure 1. The nasal congestion symptom score for intranasal treatment with xylometazoline and placebo [14].

Alpha-adrenergic receptor agonist nasal decongestants including oxymetazoline and xylometazoline present excellent ability in nasal congestion relief, for example, Barnes et al. verified the effect of intranasal xylometazoline within 15 minutes after the spray was greater than the daily administration of INMF after 28 days [5, 18]. However, α -adrenergic agonists provide quick relief by stimulating the contraction of the smooth muscles, without directly targeting the underlying inflammation. Therefore, they are recommended for a temporary alleviation of the stuffy nose, for example, in a period of having a cold. In contrast, as drugs aiming to suppress inflammation which causes nasal congestion in the first place, corticosteroids can gradually mitigate the symptoms in the long term fundamentally.

It is worth noting the potential of the combination therapy of intranasal alpha-adrenergic receptor agonists and corticosteroids. Neighbors et al. analysed the enhanced effect of the combined use of corticosteroid and oxymetazoline sprays against rhinitis symptoms compared with separate ones with no sign of induced rhinitis medicamentosa [19]. However, this strategy is seldom investigated and carried out. Further elucidation of its efficacy and possible side effects is still required [5].

5. Safety

Rhinitis medicamentosa is a result of overusing topical nasal decongestants, usually for more than a week, which in turn causes nasal congestion again [7]. Along with dryness, epistaxis, burning, and such moderate symptoms [5, 14], some other side effects may also occur as the dose goes high or in specific predispositions. Alpha-1 agonists may induce hypertension, dysrhythmias including tachycardia and so forth. Sleepiness and drowsiness are two side effects of alpha-2 receptor agonists frequently observed. [9].

Hitherto, intranasal corticosteroids (INCSs) have always been a relatively safe drug. Discomfort or burning, epistaxis, and dryness are symptoms similar to alpha agonists. Crusting, headache, sore throat and foul taste are mild symptoms but are of little prevalence. Septal perforation was reported in several isolated cases, and there was anecdotal data on temporary increased intraocular pressure [5]. The intranasal corticosteroid mometasone furoate, for instance, possesses excellent safety. Pregnant women and growing children can safely use it. The populace has verified its excellence in efficacy and safety since it has been on the market for about 24 years [16].

The nature of these drugs being intranasal sprays indicates that the nasal mucosa might be irritated due to improper ways of administration. These include mild symptoms such as discomfort resulting from burning, epistaxis, dryness, crusting, and sore throat. Hypertension, dysrhythmias, sedation and fatigue

are probable adverse effects of alpha-adrenergic receptor agonists, but rhinitis medicamentosa should draw more attention since a vicious circle of nasal congestion may be generated by overusing topical nasal decongestants indiscriminately. INCSs, however, have no harms other than the ones mentioned before and are preferred for long-term use.

6. Conclusions

Nasal congestion, inflicting inconvenience on both individuals and societies, stems from swelling from inflamed nasal mucosa. Intranasal alpha-adrenergic receptor agonists and corticosteroids as prevalent medical therapies, can be utilised in different situations due to the discrepancies in modes of action, effectiveness and potential undesirable effects. Alpha-adrenergic receptor agonists have potent effects on the amelioration of nasal congestion but imply the risk of rhinitis medicamentosa from long-term use. Corticosteroid sprays provide gradual alleviation of nasal inflammation thus improving nasal ventilation. This research further clarifies and emphasises their application scenarios and underlying reasons, facilitating the understanding of the links and differences between these two drugs. With improved explanation, patients suffering from nasal congestion may opt for the most suitable medication while the misuse of intranasal decongestants leading to the vicious cycle of rebound congestion, which is a vital issue, can be addressed. Unfortunately, according to current literature, there are still several aspects of the mechanisms of action that need clarification for better application of the sprays in the future. At the same time, investigations into their combined use provide promising prospects in nasal congestion therapy.

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Analysis of the toxicity and treatment methods of cyanide

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Abstract. Cyanide has historically been widely regarded as a highly toxic poison. In the 19th century, cyanide was used by terrorism to poison and manufacture chemical weapons. Due to occasional cyanide leakage incidents, cyanide has reappeared in people's field of vision. It has developed a great interest in how this poison is viewed and handled. This research describes cyanide in detail in terms of source, history, use, and mechanism of action. At the same time, the relationship between the toxicity and dose of cyanide, the types of diseases caused by cyanide and their signs and symptoms are also explained. In addition, some of the main existing treatments and drugs are introduced, as well as the mechanism and principle of their functioning. The significance of this research lies in the systematic understanding of cyanide, helping people deepen their understanding of its toxicity and treatment, and providing new ideas for the development of cyanide antidotes.

Keywords: cyanide, toxicity, therapy.

1. Introduction

Cyanide, an inorganic salt or organic substance with a cyano group, is a deadly xenobiotic. Cyanide can be obtained from a variety of sources, including industrial, medical, and even common household products. In addition, any material containing carbon and nitrogen may release cyanide during pyrolysis, such as many synthetic polymers or certain natural materials. Cyanide is known for its powerful toxic effects and transient onset. This highly effective and deadly poison is often used as the first choice for suicide, poisoning, assassination and terrorism. Cyanide can actually be seen everywhere in our lives. And also, cyanide is widely present in nature, and cyanide in plants is found in seeds such as bitter almonds, loquat kernels and apple kernels, as well as cassava, corn, beans and a large number of plants. Amygdalin, found in plum seeds and pits, is the most well-known food source of cyanide. Amygdalin is a cyanogenic glucoside, a sugar compound with a cyanogenic group attached, and it is also a purified form of the natural cyanide compound, amygdalin. The significant problem with intravenous amygdalin is not cyanide toxicity, but its constant excretion in the urine. Amygdalin can be metabolized to hydrogen cyanide by the enzyme glucosidase in the gastrointestinal tract.

Sources of cyanide include not only a variety of natural plants as well as industrial synthesis, but can also be released from certain compounds through chemical reactions [1]. For example, amygdalin cyanide poisoning, amygdalin is usually different concentrations of amygdalin, amygdalin obtained from certain nuts or stone kernels enters the human body with food ingestion, and cyanide can be released in the emulsifying enzyme complex when it is catabolized by B-glucosidase. In this case,

amygdalin is ingested with emulsified foods, and small doses can lead to severe poisoning or death. If amygdalin ingested from plant sources has a delayed onset of symptoms and a slower progression, it provides more time to diagnosis and intervention clinically, and is more likely to obtain favorable outcomes.

Cyanide is both widely available and readily available in various forms. One form of cyanide is highly active salts, volatile, water-soluble liquid forms of hydrogen cyanide or cyanide chloride, which can be used in many industrial applications, including chemical synthesis, electroplating, metallurgy, and as pesticides. Another common form is a chemical group called nitrile, such as acetonitrile and propionitrile. As parent compounds, they do not produce significant toxicity, but are metabolized and decomposed into cyanide under the action of the corresponding enzymes in the liver. People exposed to these organic cyanides experience delayed signs and symptoms because an incubation period of several hours passes, during which the toxin turns to form and is released. When the toxin forms and is released after an incubation period of several hours, the patient will experience delayed signs and symptoms. In addition, hydrogen cyanide gas is produced when these salts are mixed with strong acids, posing significant risks to both industrial accidents and intentional exposure. There is the form of insoluble salts such as mercury cyanide and copper cyanide, which can be found in industrial settings, especially in waste generated during mining. Although they are significantly less toxic than their salt counterparts, they can still cause considerable impact and harm to the environment by accumulating them.

Until chemicals were isolated and identified, cyanide was used as a poison. Cyanide is actually an important chemical raw material, mainly used for gold refining, electroplating, metal treatment and basic chemical synthesis, such as organic synthesis of medicine, pesticides and food additives. Cyanide can enter the human body through skin contact, respiratory inhalation, oral administration, injection and other ways. Its ingestion can lead to a high body burden of cyanide, severe symptoms, and unique toxicodynamics [2]. Cyanide exposure has the ability to cause significant social disruption, such as causing severe social unrest and public panic, a threat that requires focused public health attention and preparedness. This research will discuss the toxicity, symptoms, and treatment methods of cyanide.

2. Toxicity of cyanide

Cyanide is highly toxic and deadly, mainly due to its rapidity of action. The form of cyanide significantly affects toxic doses. Cyanide poisoning is still a major threat to human health and safety, commonly known as potassium cyanide (KCN), hydrocyanic acid (hydrogen cyanide), and sodium cyanide (NaCN).

The main effect of cyanide poisoning is caused by inducing non-competitive inhibition of the activity of chromosomal cytochrome oxidase. The mechanism of action of cyanide is that the precipitated cyanide ions combine with the trivalent iron of oxidized cytochrome oxidase in mitochondria, preventing its reduction, resulting in blockage of the cellular respiratory chain, disorders of the cellular respiratory system, and a series of reaction consequences caused by tissue cell hypoxia, such as oxygen oxidative metabolism, phosphorylation, cellular hypoxia and lactic acidosis [3]. This binding of cyanogenic ions enables almost complete inhibition of cytochrome oxidase activity, resulting in anaerobic metabolism, severe reduction in ATP production and lactic acid accumulation [1].

Iatrogenic sources are also one of the causes of cyanide poisoning. Each molecule of potent antihypertensive nitroprusside releases up to five cyanide groups, so toxicity may develop as cyanide accumulates in the body with long-term drug use. The toxicity of a substance is inextricably linked to its dose. There are certain safe limits to cyanide intake, and its lethal dose is related to a person's age, weight, physical strength, and even the amount of food left in the stomach at that time. In general, the average lethal amount of sodium cyanide is 150 mg, potassium cyanide 200 mg, hydrogen cyanide 100 mg, that is, adults oral 150-250 mg cyanide can cause sudden death. Cyanide dissociates cyano-based ions in the human body, which cause a series of biochemical reaction barriers within cells. Cyanide ions can bind to ferric iron in oxidized cytochrome oxidase in the mitochondria, hinder the reduction of ferric iron in oxidase, thereby affecting normal cell respiration, so that tissue cells cannot use oxygen in the blood, resulting in tissue hypoxia, and the body falls into a state of internal asphyxia. The central nervous

system will rapidly lose function, and then the human body will have respiratory muscle paralysis, cardiac arrest, multiple organ failure and other symptoms and rapid death.

It is estimated that the lethal dose for adults is 50-200 mg. In fact, the lethal dose of cyanide is actually relatively high compared to other potential chemical weapons such as botulinum toxin. Its mechanism of action is the rapid diffusion of cyanide into tissues and irreversible binding to the target site. Symptoms of intravenous and inhaled cyanide occur most rapidly, while apparent toxicity from ingestion is delayed by several minutes until a certain concentration has accumulated in the blood. Because acute cyanide toxicity lacks characteristic symptoms and lacks a reasonable index of suspicion, it is often easily confused with other poisoning syndromes or missed. Victims may have bitter taste of almonds, but only about 40-60% of the population has the genes needed to detect this smell, which is not a reliable marker. A quick indicator of cyanide is to visualize retinal arteries and veins in red. This is due to an abnormal increase in poor oxygen extraction oxygen in the intravenous supply and more pronounced red. Lactic acid concentration can be used as a marker for the severity of cyanide poisoning. Cyanide concentrations are used only to confirm exposure, and patient care should be based on a suspicion index and clinical presentation.

The cyanide poisoning is a metabolic acidosis, due to the transformation of aerobic metabolism to anaerobic metabolism, resulting in a significant increase in lactic acid concentration, and severe hyperanion gap acidosis follows. Cyanide induces cellular hypoxia, leading to oxidative stress and lipid membrane peroxidation. The long-term effects of cyanide exposure include significant complications such as neurological disorders.

3. Symptoms of cyanide poisoning

Some clinical signs and symptoms may be associated with cyanide poisoning [1]. Clinical manifestations are usually initially hyperpnea and central nervous system irritation. Thus, there are different late symptoms such as vomiting and bradycardia. Long-term consumption of cyanated substances can also indirectly cause some related syndromes, such as optic nerve atrophy, nutritional ataxia neuropathy and other neurological diseases. The symptoms and onset time of cyanide poisoning are closely related to the physicochemical structure of cyanide. The onset time of cyanide in different phases varies by several times, with gaseous hydrocyanic acid being a few seconds, while solid or liquid cyanide is a few minutes, and cyanide-producing compounds taking several hours. Therefore, effective treatment of cyanide poisoning requires a race against the clock.

4. Analysis of treatment methods

The pathway of endogenous cyanide metabolism occurs mainly in the liver, and sulfur and cyanocobalamin's reaction metabolism is normally encountered in low concentrations of cyanide, and some enzymes act to catalyze the conversion of cyanide to thiocyanate. These enzymatic pathways, while very effective, are not sufficient to work effectively in cases of poisoning due to sulfur donor depletion. In cases of acute intoxication, endogenous. The pathways of cyanide metabolism are rapidly overwhelmed and the depletion of conjugated substrates allows cyanide accumulation and progression of toxic effects.

Diagnosing cyanide poisoning is difficult, especially for emergency physician or rescue worker at the scene, which remains a challenge on their capability of the fast and 'on the scene' suspect or diagnosis of cyanide poisoning. The difficulty of cyanide poisoning clinical diagnosis is related to the poisoning and the history of aetiology. For example, in fire victims, carboxyhemoglobin, the identifier of carbon monoxide poisoning is easy to measure and durable, while cyanide toxin identification is difficult due to its short half-life and poor stability [3]. However, asphyxia syndrome can be induced by carbon monoxide or cyanide, suggesting that clinicians cannot confirm the diagnosis by examination of the exposed site. Therefore, cyanide poisoning caused by smoke inhalation, and the patient's signs and symptoms related to cyanide exposure or carbon monoxide exposure remain to be distinguished and clarified [3]. Cyanide poisoning can cause adverse effects in addition to inhalation or ingestion of adequate doses of cyanide. Because there are many sources of potential cyanide exposure and there is

no specific cyanide assay suitable for acutely poisoned patients, the diagnosis of cyanide is based on a history of cyanide exposure. Supportive measures can be used for the treatment of cyanide, as well as specific and effective antidotes available.

Cyanide poisoning also has specific symptoms that aid in its presence and diagnosis. For example, hydrocyanic acid smells bitter almonds, and when this taste is present, this finding has high diagnostic value. However, there is a high probability that no one noticed the bitter almond smell, or was hindered by other odors such as a strong smoke smell. Although the symptoms of cyanide or cyanide compound poisoning have been described in detail, they remain difficult to diagnose due to their complexity and diversity. There are also symptoms that can only be made after a post-mortem and are proven by prior post-mortem attempts. In patients without an overt cyanide poisoning syndrome, acid lactic acid acidosis with normoglycemia, convulsions, and tachypnea with cyanosis at the time of death is likely to be caused by cyanide poisoning. Blood cyanide concentration cannot be measured on the spot and results can be obtained, so it is still not very useful and helpful in emergency situations. In addition, the determination and diagnosis of cyanide concentration in the blood is still correspondingly difficult. Due to the instability of cyanide in the blood, it will quickly disappear and decompose, so the sooner the better when determining and analyzing, otherwise its accurate concentration cannot be obtained, and it is easy to underestimate its severity. The lethal dose of human cyanide and its corresponding blood cyanide concentration vary according to the human constitution, so it cannot be accurately defined [3]. Because the clinical manifestations, diagnosis, and prognostic factors of cyanide poisoning are unclear, emergency management and optimal treatment options for cyanide poisoning remain challenging and subject to discussion. The survival prognosis of cyanide poisoning is related to the form, route, quantity, type of poisoning, and speed of treatment.

Although cyanide has many possible sources of exposure and exposure to ingestion routes in everyday life, life-threatening acute cyanide poisoning is rare [1]. Cyanide is one of the few toxic drugs with a specific antidote. In fact, cyanide in life will not have too much impact on the human body as long as it is properly handled. Heating food containing cyanide can effectively remove the cyanide and evaporate it. In addition, the common sodium cyanide and potassium cyanide are a solid crystal or powder in the normal state, which is more stable and easier to control, and will not have harmful effects on the environment or the human body in this case. Cyanide is not a cumulative poison, it is easily degraded or eliminated, and the relevant technology has been perfected. The microorganisms in the natural water body have a strong purification effect on low concentrations of cyanide, which can be converted into non-toxic compounds, and the human body also has a certain degradation ability to the cyanide, and there is also a corresponding detoxification mechanism for a small amount of cyanide that has not reached the toxic dose into the body. High concentrations of cyanide can be artificially degraded to safe concentrations with chlorine or hydrogen peroxide. Therefore, the conditions for cyanide poisoning are more limited and harsher, and countries have specific qualified requirements for cyanide content in food and water. If unfortunately cyanide poisoning does not mean death, there are now a variety of first aid methods, such as skin contact can be washed, mistakenly taken can induce vomiting for medical treatment, and cyanide also exists specific drugs to treat.

Among all the anions cyanide is extremely poisonous to mankind and affects many physiological functions [4], and different detection methods have been used to detect cyanide, as shown in Figure 1. Cyanide poisoning targets the neurological, cardiovascular, and respiratory systems, while inducing deep metabolic disorders leading to permanent neurological dysfunction ranging from various extrapyramidal syndromes to stopping hypoxic vegetative states [3]. There are several treatment options for cyanide poisoning. The goal of supportive care for cyanide-induced life-threatening conditions is to reverse organ failure, such as coma, seizures and cardiovascular failure. However, it is effective only in the early stages of cyanide poisoning, and purification depending on the assumed absorption pathway is also an effective method.

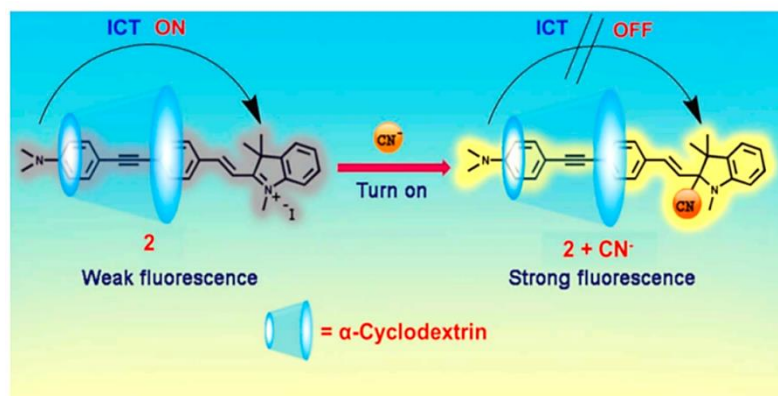


Figure 1. The cyanide sensing mechanism [4].

There are also specific treatments, such as management of the antidote cyanide. There are different types of cyanide antidote-moglobin inducers, including amyl nitrite, sodium nitrite, and 4-dimethylaminophenol, sodium thiosulfate, dicobalamin, and hydroxocobalamin [3]. Besides, the only cyanide antidote available in the United States is a three-component cyanide ampero supplement containing sodium thiosulfate, amyl nitrite, and sodium nitrite [5].

The mechanism of action of methemoglobin inducers is mainly by converting ferrous hemoglobin to ferric iron, subject globin formation may then compete with cytochrome oxidases for cyanide binding and experimental data suggest that mechanisms other than methemoglobin induction are also important antitoxic effects of these drugs [3]. Rhodanese and sulfur transferase are able to convert cyanide to less toxic thiocyanate in the presence of its natural substrate---thiosulfate, which can be eliminated in tea urine. Because it is an effective but slow treatment, it cannot be used as a first-line cyanide antidote [3]. Cobalt has a high affinity for cyanide, and each molecule can bind two cyanides. Hydrocobalamin, both experimental and clinical, is generally a potent cyanide antidote. Hydrocobalamin binds to cyanide and the form cyanocobalamin is non-toxic compounds, which are then excreted in the urine. Its negative inotropic effect on cyanide is rapid. However, this methemoglobin inducer may induce serious side effects, including cardiovascular collapse, which may be more severe in the absence of cyanide and need to be used with caution [3]. Sodium nitrite can cause methemoglobinemia and severe hypotension, leading to potentially fatal reductions in blood oxygen-carrying capacity, as well as shock and death, and must be monitored at all times in a hospital management setting [3]. In addition, thiocyanates have antithyroid activity, and dicobalamin EDTA often has harmful side effects on the cardiovascular aspect and it is also poorly tolerated. The mainstay of treatment is a 100% oxygen and cyanide-specific antidote, which aims to reduce the amount of cyanide available for binding to respiratory enzymes in cells [6].

The mechanism of action of nitrite is mainly the induction of methemoglobinemia. Since methemoglobin has a greater affinity for cyanide than the trivalent iron portion of cytochrome oxidase, it can be used to exchange cyanide from respiratory enzymes to enable the enzymes to perform their normal functions [1]. In the absence of methemoglobinemia, sodium nitrite can be detoxified by local vasodilation or other mechanisms. Sodium thiosulfate acts as a sulfhydryl donor, reversibly binding to cyanide in the extracellular space to form thiocyanate that is minimally toxic and excreted by the kidneys, thereby enhancing cyanide clearance. The only significant adverse effects of sodium thiosulfate were rare hypersensitivity reactions and infusion rate-dependent hypotension. Long-term exposure to thiocyanates can cause toxicity due to the equilibrium between thiocyanate and cyanide. For patients presenting within 1 hour of cyanide ingestion, oral and gastric lavage and administration of activated charcoal may be considered to attempt cyanide recovery. Despite the low level of binding of activated charcoal to cyanide, its administration has been shown to be effective due to the relatively small lethal dose of cyanide.

Ventilation with 100% oxygen increases tissue oxygen supply, an important component of supportive care and synergistic with antidotes. Oxygen may contribute to increased respiratory excretion of cyanide,

restore cytochrome oxidase activity by replacing cyanide, stimulate activation of other oxidizing systems, and may indirectly promote rhodinase metabolism by catalyzing cyanide. Hemodialysis is advocated in certain cases of cyanide poisoning; This will be especially beneficial in the face of worsening acidosis and kidney failure while leading to thiosulfate buildup and inducing additional toxicity of its own. Due to various human activities and informal waste disposal, the generation and legacy of cyanide waste has become an increasingly common problem today, mainly including: coke plants, automobile manufacturing, oil refining and mining [7]. The intense toxicity of cyanide and the environmental concerns and exposure risks associated with its industrial use continue to generate interest in rapid, safe and effective cyanide disposal methods [8].

Many efforts have been made to develop safe and effective detoxification and utilization techniques to reduce the harmful effects of cyanide on public health and the environment [9]. The study of potential antidotes is complicated by the fact that the pharmacokinetics of individual drugs can change due to cyanide toxicity, making it difficult to predict the efficacy and toxicity of antidotes. Because cyanide inhibits many enzyme systems, many potential detoxification mechanisms remain untapped. Exogenous administration of enzymes or substrates commonly involved in cyanide metabolism and detoxification may prove beneficial. Some studies have reported treatments such as carrier erythrocytes containing rhodinase and thiosulfate to reverse cyanide toxicity. When assessing the usefulness of antidotes, determining whether they reduce or even eliminate the need for incremental supportive care is one of the main points [3]. Studies have shown that antidotes with first-line therapeutic potential are being developed in terms of fewer side effects and are safer. Future research should move towards finding faster, safer, more effective, and better tolerated cyanide toxicity treatments and antidotes [10].

5. Conclusion

This research systematically analyzed the toxicity and mechanism of action of cyanide, as well as its symptoms and treatment methods. Although cyanide is a highly toxic and fast-acting poison, its toxicity is closely related to the dose, and toxicity will only occur when a certain dose is reached. Even if cyanide has multiple exposure routes and ways to enter the human body, within a safe dose, cyanide can be metabolized by our own detoxification mechanism, and there are multiple rescue measures for misconduct. Even if it is unfortunate to be poisoned, there are now a variety of effective treatment channels and drugs, and its mortality rate has been reduced year by year. However, there are still great difficulties in the diagnosis of cyanide poisoning, and its antidote still has great side effects and limited use conditions, so the research of rapid and effective diagnosis methods and efficient and safe antidotes is the future development trend.

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The current status and prospects of research on orphan G protein-coupled receptor

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Abstract. This review provides an overview of the current status and prospects of research on orphan G protein-coupled receptors (oGPCRs). It begins by discussing the classification and structure of GPCRs and then delves into the methods used to identify and characterize ligands for oGPCRs, including ligand screening strategies, correlation analysis, functional analysis techniques, and interaction evaluations. The review further examines the functionality and de-orphanization research of specific oGPCRs, such as GPR35, GPR55, GPR75, and GPR88. It discusses their basic information, signalling pathways, research on endogenous agonists, and their associations with diseases and physiological activities. Finally, the review concludes with a discussion of future directions and prospects for research on oGPCRs, emphasizing the importance of understanding receptor functionality and signalling pathways for drug development

Keywords: Orphan G Protein-Coupled Receptor, Signal pathway, Ligand.

1. Introduction

G protein-coupled receptors (GPCRs) constitute a broad classification encompassing a substantial cohort of membrane receptors, orchestrating their physiological impacts via G proteins [1]. The native ligands for nearly 210 of these GPCRs have been characterized, while approximately 140 others retain undefined endogenous ligands, thereby denoted as orphan G protein-coupled receptors (oGPCRs). A large number of oGPCRs have not been functionally clarified and so remain of great research value. The discovery of endogenous ligands of oGPCRs can significantly advance the exploration of their physiological roles and pathological implications, thereby offering fresh theoretical underpinnings for investigating the mechanisms underlying associated diseases and fostering drug development efforts targeting these receptors. For examples Doxycycline, a potential drug for cancer therapy, is potentially active against several oGPCRs associated with tumour growth and metastasis [2]. Studies conducted in this domain have garnered considerable scholarly interest.

2. Orphan G protein-coupled receptors

2.1. Structure of G protein-coupled receptor

Based on the structural attributes of GPCR protein molecules, they can be broadly partitioned into three domains: intracellular region, transmembrane region, and extracellular region. The intracellular domain engages in recognizing G protein signaling molecules and binding with pertinent signaling moieties. The transmembrane domain constitutes the foundational framework of GPCR protein molecules, facilitating signal transduction and ligand binding throughout the reaction cascade. The extracellular domain predominantly assumes the role of recognizing signaling molecules [3].

The extracellular domain of GPCR protein molecules comprises three extracellular loop segments and the N-terminal region. The extracellular component of GPCRs exerts a stabilizing influence on the receptor's spatial conformation, facilitated by the presence of two highly conserved cysteine residues capable of forming a disulfide bond. Additionally, glycosylation frequently occurs on the extracellular loops of GPCRs.

The transmembrane domain of GPCR protein molecules is composed of seven transmembrane helices, categorized into two segments: helices I-IV and helices V-VII. Helices II, III, VI, and VII principally compose the substrate-binding pocket of GPCRs, serving the primary purpose of substrate recognition. Conversely, helix V predominantly engages in substrate molecule binding and the conformational adaptations required for distinct substrates, thereby exerting additional modulation over the receptor's diverse functionalities [4].

The intracellular portion of the GPCR protein molecule encompasses the helical conformation of the eighth segment positioned within the cell, the three-segment intracellular loop domain, and the intracellular segment of the GPCR's carboxy-terminal region [5]. The initial two out of the three intracellular loops are comparatively brief and lack distinct biological functionalities, in contrast to the third intracellular loop, which exhibits greater dimensions. Its principal role involves binding with downstream effector proteins to facilitate signal transduction [4].

2.2. Regulating Pathways

The G protein pathway represents a frequently employed signaling cascade in GPCR-mediated signal transduction. The G protein associated with GPCRs is composed of α , β , and γ subunits. In the absence of ligand stimulation, the α subunit of the G protein remains bound to GDP, maintaining an inactive state. Upon activation of the receptor by a ligand, the G protein undergoes a conformational alteration. GTP replaces GDP on the α subunit, leading to its dissociation from the β and γ subunits. The α subunit transitions into an active state, while the unbound β and γ subunits gain the capacity to activate their respective effectors [6]. Upon activation, the α -subunit engages with the respective enzyme or ion channel, initiating a sequence of cascading reactions that modulate downstream signal transduction pathways, thereby regulating associated physiological processes.

Based on the downstream signals and the similarity of the α -subunits, the α -subunits can be classified into four groups: Gs, Gi/o, Gq/11, and G12/13 [7].

Upon activation of GPCRs coupled to Gs, Gs proteins activate intracellular adenylyl cyclase (AC), which catalyzes the cyclization of ATP into cyclic AMP (cAMP), thereby regulating cellular metabolism as well as the expression of specific genes. cAMP can be degraded by intracellular phosphodiesterase to 5'-AMP, which terminates the signalling transduction. In contrast, GPCR coupled to Gi/o inhibits cAMP synthesis by inhibiting AC activity upon activation [6].

2.3. Related Diseases

Several orphan GPCRs have undergone investigation and have been linked to particular diseases. Notably, certain oGPCRs have exhibited potential associations with cancer metastasis and progression; for instance, GPR35 has demonstrated a robust association with conditions like lung cancer, while heightened GPR55 expression levels have shown a positive correlation with the malignancy grade of glioblastoma.

Moreover, it has been demonstrated that orphan GPCRs are implicated in specific neurological disorders. As an illustration, GPR85, which researchers have indicated via animal experiments to play a role in brain size determination, the modulation of animal behavior, and a potential association with schizophrenia, exhibits overexpression, resulting in various cognitive and behavioral abnormalities in individuals [8]. These abnormalities encompass diminished social aptitude and disruptions in motor-sensory coordination. In a separate investigation, GPR85 is identified as an etiological factor in schizophrenia and exerts a negative regulatory influence on hippocampal adult neurogenesis as well as neurogenesis-related processes tied to learning and memory [9].

Certain immune system disorders are also subject to modulation by orphan GPCRs. Evidence suggests that GPR18 is linked to immune cell migration and inflammatory responses, implying a potential role in immune-related diseases [10]. Furthermore, the de-orphanized receptor EDG1 actively participates in immune system regulation, with particular significance in lymphocyte migration and activation processes. EDG1 is expressed on immune cell types like T cells and B cells, where it exerts control over their activation, migration, and circulation by binding to S1P.

3. Ligands of oGPCRs

The identification of endogenous ligands for orphan receptors remains incomplete, constituting a central research challenge. The primary objective in the quest for these ligands is to elucidate the functions and biological roles of these receptors, a pursuit imbued with significant biological and medical implications. oGPCRs are typically pivotal in orchestrating a diverse array of intracellular physiological processes, including but not limited to cellular growth, differentiation, and immune responses. The discovery of their respective ligands holds the potential to shed light on the receptor's role in cellular signaling and physiological regulation. Moreover, the identification of ligands for orphan receptors presents promising avenues for the development of novel drug targets, thereby offering prospects for the creation of innovative therapeutics spanning a broad spectrum of ailments, including cancer, autoimmune disorders, and neurological conditions.

3.1. Workflow

Presently, the most widely employed approach for the discovery of ligands for orphan receptors involves the utilization of an orphan receptor strategy. In this strategy, the orphan receptor serves as a 'bait' to isolate novel ligands and elucidate their functional roles. The fundamental procedure entails the transfection of the cloned orphan receptor gene into vector cells, which subsequently express the corresponding receptor. These transfected cells are then exposed to tissue extracts containing potential natural ligands for the receptor. The interaction between the ligand and the receptor triggers the generation of a second messenger, and alterations in the second messenger within the vector cells are measured as a parameter to monitor the purification of ligands specific to the orphan receptor [11]. High-throughput screening methodologies are employed to evaluate a large number of candidate ligands in the quest for ligands suitable for orphan receptors.

3.2. Several Common Detection Techniques

The primary assay technique presently employed for the identification of endogenous ligands for oGPCRs involves the analysis of variations in intracellular Ca^{2+} concentrations subsequent to phospholipase C (PLC) activation. This method is favored due to its stability, amenability to automated detection, and high throughput capacity. PLC, an enzyme that typically interacts with receptor proteins on cell membranes, becomes activated when the target receptor binds to its ligand, thereby initiating dynamic changes in intracellular Ca^{2+} concentrations. Receptor-ligand interactions are evaluated by monitoring fluctuations in intracellular Ca^{2+} levels to unveil ligands that correspond to oGPCRs. Several commonly employed assays for this purpose are delineated below.

Fluorescence imaging reader method (FLIPR) It is an experimental technique commonly used in bioscience research to monitor and measure the fluorescence emission of cells or biomolecules, is commonly used to study biomolecules within cells and can provide information about their localization;

Cell visualization It is an experimental technique employed to observe and investigate various aspects of living cells, including their structure, function, behavior, interactions, and more. This method offers an intuitive visual comprehension of specific biological processes occurring within cells;

Jellyfish luminescence is a scientific method that leverages bioluminescent reactions to investigate cellular activity and molecular interactions. This approach relies on the luminescent properties of calcium-sensitive receptor proteins, allowing for the real-time monitoring and visualization of cellular processes;

Using activation of the phospholipase A2 (PLA2)-Ca²⁺ pathway to identify ligand The radioactivity associated with the radioligand bound to orphan G protein-coupled receptors (oGPCRs) was quantified using the arachidonic acid release assay.

4. Functionality and De-Orphanization Research of several oGPCRs

4.1. GPR35

4.1.1. *Basic information.* G protein-coupled receptor 35 (GPR35) is a class A, rhodopsin-like orphan GPCR that has been implicated in a variety of diseases and physiological activities, but for which no endogenous ligand has been identified.

4.1.2. *Signaling pathway.* Its signaling pathway is widely distributed, and it can couple with Gai/o and Gα13 while recruiting β-arrestin [12]. Among these, GPR35/Gai/o promotes the phosphorylation of c-Jun amino-terminal kinase and extracellular signal-regulated kinase 1/2, thereby participating extensively in physiological activities such as cell migration and immune regulation [13]. It also activates peroxisome proliferators, receptor γ coactivator-1α, and promotes lipid metabolism, thermogenesis, and the expression of anti-inflammatory genes [14]. GPR35/Gα13 impacts actin regulation, cytoskeletal rearrangement, and vascular remodeling through Ras homolog A/Rho kinase signaling. GPR35 can also activate Gai/o and Gα13 signaling, and it translocates to the outer mitochondrial membrane where it binds to ATPase inhibitory factor 1, thus preventing ATP loss during ischemia [15].

4.1.3. *Research related to endogenous agonists.* Agonists are molecules that induce receptor activation by binding to the receptor, such as KA(Kynurenic Acid), lysophosphatidic acids (LPA), chemokine 17 (CXCL17), 5-hydroxyindole acetic acid (5-HIAA) and the ethyl acetate extract of thyme, which are prominent among the endogenous substances [12, 16]. These endogenous substances are currently being investigated as potential agonists for GPR35. Nonetheless, these candidates are not without issues that have prompted debates, thereby complicating the precise identification of the authentic endogenous agonist.

The debates regarding KA as the primary endogenous agonist of GPR35 stem from several factors: (1) To elicit GPR35 activation in vitro, KA concentrations often need to be elevated to the millimolar range, whereas human plasma levels of KA typically remain within the nanomolar range. Nevertheless, during inflammatory or pathological conditions, local KA concentrations can rise to millimolar levels. (2) KA also triggers the activation of other receptors such as NMDAR, α7nAChR, and AhR. Its in vivo effects involve multiple targets and intricate regulatory networks, posing challenges in dissecting its specific association with GPR35. (3) KA exhibits notable selectivity for rodents, with its potency for mouse and rat GPR35 being 40-100 times higher than for human GPR35. This rodent selectivity complicates the confirmation of its precise mechanism [12].

Lysophosphatidic acid (LPA) and chemokine 17 are also frequently investigated as potential endogenous agonists of GPR35. However, it is noteworthy that LPA functions as a biased ligand for GPR35. Specifically, LPA activates Gαq signaling pathways downstream of GPR35, while not affecting Gai/o, Gα13, or β-arrestin signaling. Consequently, LPA does not induce GPR35 activation in standard GPR35 ligand screening assays [17].

In vitro GPR35 ligand screening assays have indicated agonist activity for the second messenger cGMP and certain tyrosine metabolites. However, these ligand screening assays did not encompass GPR35-related signaling mechanisms or functionalities, and their findings have not received validation from other investigations [18].

4.1.4. GPR35 is associated with disease. GPR35 is inflammatory bowel disease (inflammatory bowel disease, IBD) risk of genes, and ulcerative colitis (ulcerativecolitis, UC) and Crohn's disease (Crohn's disease, CD) and so on. GPR35 plays a protective role in the intestinal tract through various signaling mechanisms: Tsukahara et al. found that PA/GPR35 promotes fibronectin, integrin-5 expression and ERK1/2 phosphorylation, thus promoting colonic mucosal repair [12]. Some research found CX3CR + macrophages of LPA/GPR35 promoting TNF alpha, aldosterone hydroxylase 11 members of the family of the cytochrome P450 b1 (cytochromeP450b1 Cyp11b1) expression, thus maintaining the steady state [19]. Sun et al. found that KA/GPR35 inhibits the over-activation of NLRP3 inflammasome and reduces the secretion of IL-1 β when stimulated by lipopolysaccharides (LPS) [20].

GPR35 is associated with colorectal cancer (CRC), gastric cancer, breast cancer and other cancers, and the data from The Cancer Genome Atlas (TCGA) and The Genote-Tissue Expression (GTEx) databases were found to be positive Compared with normal tissues,GPR35 expression was upregulated in colorectal, gastric, and pancreatic cancers, but unchanged in ovarian and breast cancers. More importantly, clinical data show that GPR35 overexpression is associated with poor prognosis in patients with colorectal cancer, non-small cell lung cancer, and gastric cancer, while GPR35 expression level is associated with tumor differentiation, stage, and metastasis in breast cancer and gastric cancer [12]. Meanwhile, some research shows that the Neuroglobin plays as tumor suppressor by disrupting the stability of GPR35 in colorectal cancer [21].

The components of GPR35 related signaling pathways and their functional roles in various organs and tissues have been obtained. The gradual identification and identification of agonists meet the need for further exploration of the function of GPR35. Further studies are needed to further understand the mechanism of action between agonists such as KA and lysophosphatidic acid and GPR35, which will lay a foundation for the development of its targeted drugs.

4.2. GPR55

4.2.1. Basic information. GPR55 is classified as a member of the class A retinoid-like receptors. Lysophosphatidylinositol (LPI) serves as a potent agonist for GPR55. Furthermore, GPR55 exhibits specific binding affinity for certain cannabinoid ligands, leading some researchers to propose its categorization as a cannabinoid type 3 receptor. However, numerous unresolved issues exist.

4.2.2. Signaling pathway. The physiological roles of GPR55 are currently not well understood. However, it is evident that GPR55 exhibits widespread distribution and is implicated in various physiological activities through its associated signaling pathways. Notably, GPR55 has been shown to couple with G12/13 and Gq proteins, thereby initiating a sequence of downstream signaling cascades [22].

4.2.3. Function. Its functionalities are multifaceted, and existing research has demonstrated its potential as a novel anti-diabetic target that could have favorable impacts on β -cell function and quality. Furthermore, empirical evidence indicates that the LPI/GPR55 axis contributes to pro-carcinogenic processes encompassing cell proliferation, differentiation, migration, invasion, and metastasis. This involvement is facilitated through G12/13 and Gq signaling pathways and is altered in various cancer cell types. Additionally, both GPR55 itself and its bioactive lipids are being explored as potential biomarkers for cancer diagnosis. Notably, an association has been established between elevated or heightened GPR55 expression and the aggressiveness of specific tumors, including acute myeloid leukemia, uveal melanoma, low-grade gliomas, and renal cancer. In the context of studies related to depression, investigations have revealed that the down-regulation of GPR55 expression in the

hippocampus might mediate depression and anxiety-like phenotypes induced by chronic social defeat stress (CSDS). Conversely, the activation and up-regulation of GPR55 could potentially yield anti-inflammatory and subsequent neuroprotective effects, offering a prospective avenue for depression treatment. Remarkably, the GPR55 agonist O-1602 has shown significant efficacy in mitigating CSDS-induced depression and anxiety-like behaviors, along with the amelioration of neuroinflammatory and neurogenic deficits in the hippocampus [23].

4.2.4. Research related to endogenous agonists. Lysophosphatidylinositol (LPI) exerts potent agonist activity on GPR55. It also exhibits specific binding affinity to certain cannabinoid ligands, prompting some researchers to consider it as a potential ligand for the cannabinoid type 3 receptor. However, this viewpoint is accompanied by unresolved issues. Notably, GPR55 lacks homology to the pro-CB1 and CB2 (cannabinoid receptor 1 and 2) receptors, and traditional cannabis binding sites are not identified [24]. The question of whether cannabis acts as an antagonist or agonist remains complex. Research has indicated that endogenous cannabinoids can function as both antagonists and activators of GPR55. Among these, analogs of cannabidiol have been identified as potent agonists of the GPR55 cannabinoid receptor, demonstrating EC₅₀ values of 13, >30,000, and >30,000 nM for GPR55, CB1, and CB2 receptors, respectively. In vitro studies have revealed that this compound inhibits mouse osteoclast formation, stimulates osteoclast polarization and resorption, triggers Rho and ERK1/2 activation, and impedes the migration of human neutrophils.

Furthermore, a relevant investigation published in the journal “Nature” demonstrated that curcumin activates GPR55, leading to the initiation of serum response element and serum response factor-mediated transcription. This activation is subsequently restrained by Rho kinase and GPR55 antagonists. Both the methoxy and heptadienone functionalities of curcumin are essential for GPR55 activation, with residue F190^Δ5.47 within the GPR55 structure playing a significant role in curcumin interaction. Notably, GPR55 antagonists hinder the curcumin-induced secretion of glucagon-like peptide-1 in GLUTag cells [23].

4.2.5. GPR55 and cancer. Many GPCRS have been shown to be associated with the pathogenesis of various cancers, including promoting cancer cell proliferation, evading immunity, inducing angiogenesis, and promoting invasion and metastasis. Studies have confirmed that the endogenous ligand LPI of GPR55 can promote cell mitosis [25]. Proto-oncogene Ras induces malignant transformation of epithelial thyroid cells and fibroblasts, resulting in the synthesis and release of LPI, and LPI, as a mitogen of these cells, can promote the proliferation of malignant cells, suggesting that LPI is involved in the occurrence and development of RAS-dependent tumors [26].

4.2.6. Summary of GPR55 features. Current studies have confirmed that LPI is the endogenous natural ligand of GPR55, and due to GPR55’s sensitivity to cannabinoids, GPR55 can explain some mechanisms that are not mediated by traditional cannabinoid receptors CB1 and CB2. Therefore, the physiological and pathological states related to LPI, including cell proliferation and differentiation, cancer, cardiovascular diseases, etc., as well as the physiological and pathological states related to cannabinoids, including inflammation and gastrointestinal diseases, are of great significance for further study of GPR55.

4.3. GPR75

4.3.1. Basic Information and Functions. GPR75 is categorized as a class A retinoid-like Gαq protein-coupled receptor. Recent investigations have unveiled the interactions between GPR75 and ligands such as CCL5 and 20-HETE [27]. On the a GPR75 is categorized as a class A retinoid-like Gαq protein-coupled receptor. Recent investigations have unveiled the interactions between GPR75 and ligands such as CCL5 and 20-HETE . On the anatomical scale, GPR75 exhibits a widespread expression across various tissue systems. Within the central nervous system, GPR75 is detectable in distinct brain regions,

including the retina, cerebral cortex, thalamus, and hippocampus. Within the cardiovascular system, GPR75 manifests its presence in tissues such as vascular smooth muscle cells and the heart. Moreover, GPR75 expression has been identified in tissues like pancreatic islets and the prostate. Anatomical scale, GPR75 exhibits a widespread expression across various tissue systems. Within the central nervous system, GPR75 is detectable in distinct brain regions, including the retina, cerebral cortex, thalamus, and hippocampus. Within the cardiovascular system, GPR75 manifests its presence in tissues such as vascular smooth muscle cells and the heart. Moreover, GPR75 expression has been identified in tissues like pancreatic islets and the prostate.

4.3.2. Research related to endogenous agonists. CCL5 is classified as an inflammatory chemokine belonging to the CC class subfamily. It binds to CC class chemokine receptors such as CCR1, CCR3, and CCR5. It has been suggested that CCL5 may potentially interact with the orphan receptor GPR75. Studies conducted on cells overexpressing GPR75 and those endogenously expressing GPR75 have indicated that CCL5 elicits intracellular IP₃ accumulation, raises Ca²⁺ levels, and triggers the activation of protein kinase B (PKB/AKT) and mitogen-activated protein kinase (MAPK) signalling pathways [27]. Interestingly, some investigations have indicated lower expression levels of CCR1, CCR3, and CCR5—receptors for CCL5—while GPR75 exhibited significant expression. Silencing GPR75 expression effectively obstructed the Ca²⁺ increase and pro-insulin secretion induced by CCL5. Furthermore, knockdown of GPR75 expression impeded the CCL5-induced phosphorylation of AKT, GSK3 β , and ERK. However, there exists some controversy regarding the potential interaction between CCL5 and GPR75, and no direct binding or interaction between CCL5 and GPR75 has been definitively established.

20-HETE, a vasoactive metabolite derived from the metabolism of arachidonic acid catalyzed by the CYP4A/4F enzymes within the cytochrome P450 enzyme family, has been identified as having significant physiological impact. A study conducted in 2017 unveiled the expression of GPR75 within the vascular system and disclosed the activation and binding of 20-HETE to this receptor. The investigation further confirmed that, in vascular smooth muscle cells, 20-HETE engenders vascular endothelial dysfunction and stimulates smooth muscle cell contraction via a GPR75-dependent mechanism. More recent inquiries have illuminated the modulation of the interaction between 20-HETE and GPR75 by CCL5. Notably, treatment with CCL5 substantially suppressed the 20-HETE-triggered escalation of intracellular Ca²⁺ concentration and IP-1 accumulation, concurrently thwarting the recruitment of β -arrestin initiated by 20-HETE. These findings strongly suggest distinct binding sites for GPR75 in 20-HETE and CCL5, whereby CCL5 appears to act as a competitive inhibitor by binding GPR75 as a low-affinity ligand, thereby impeding the activation of GPR75 by 20-HETE.

4.3.3. GPR75 and Cancer. Studies have shown that 20-HETE can activate GPR75 and affect signaling pathways in prostate cancer cells [28]. GPR75 can enhance the phosphorylation of EGFR, NF κ B, AKT, and p38 through treatment with 20-HETE. On the other hand, CCL5 may act as a competitive inhibitor of 20-HETE by binding to GPR75. These findings indicate that GPR75 and the 20-HETE/GPR75 signaling pathway could have implications in cancer development. Further research is needed to explore the precise involvement of GPR75 in cancer and its potential as a therapeutic target.

4.3.4. Summary of GPR75. In summary, GPR75 is a class A retinoid-like G α_q protein-coupled receptor that exhibits widespread expression in various tissue systems, including the central nervous system, cardiovascular system, pancreatic islets, and prostate. Studies have identified potential interactions between GPR75 and ligands such as CCL5 and 20-HETE. CCL5, an inflammatory chemokine, has been suggested to bind to GPR75, although the evidence for direct binding is inconclusive. 20-HETE, a vasoactive metabolite, has been found to activate GPR75 and impact signaling pathways. These findings indicate potential involvement of GPR75 in various diseases, including cancer, where 20-HETE/GPR75 signaling may play a role. Further research is needed to better understand the exact functions of GPR75 and its potential as a therapeutic target in cancer and other conditions.

4.4. GPR88

4.4.1. Basic Information and Functions. GPR88 is categorized as a class A G protein-coupled receptor. Its protein expression is detectable in the striatum during early embryonic development and gradually increases as development progresses in the striatum, olfactory bulb, nucleus ambiguus, amygdala, and neocortex, maintaining this pattern into adulthood. However, GPR88 expression is notably discrete in the spinal cord, pons, and medulla. Moreover, GPR88 exhibits transiently elevated expression in certain peripheral tissues, such as the adrenal cortex and cochlear ganglia, while being relatively less expressed in tissues like the retina and spleen.

4.4.2. Research related to endogenous agonists. 2-PCCA represents the first potent small molecule agonist for GPR88 [29]. In cells expressing GPR88, it exerts a GPR88-mediated concentration-dependent inhibition of isoprenaline-stimulated cAMP accumulation. It operates through Gi coupling and does not trigger calcium mobilisation. Investigations have demonstrated the adaptability of the aniline portion of 2-PCCA to various modified sites. In experimental settings, 2-PCCA diminishes locomotor activity in rats and exhibits the potential to mitigate methamphetamine-induced hyperactivity. However, it does not demonstrate the ability to counteract methamphetamine's behavioral effects, encompassing alterations in methamphetamine-induced hyperactivity and discriminative stimulus effects. In summary, 2-PCCA establishes a foundational platform for further investigations and the refinement of GPR88's in vivo functionality.

2-AMPP has also demonstrated agonistic properties towards GPR88 [29]. Through the design of a series of derivative compounds, researchers have identified that structural modifications to 2-AMPP can lead to enhanced activity. Specifically, replacement of the hydroxyl group at position A results in a twofold increase in activity, and the presence of the phenyl group at position B is pivotal for its activity. These discoveries hold significance for refining the model and facilitating deeper exploration into the structural characteristics and specificity of the GPR88 receptor.

4.4.3. GPR88 and related disorders. GPR88 has been implicated in various physiological and pathological processes, including hypertension, alcohol use disorder, and anxiety. In the context of hypertension, GPR88 expression is lower in the aorta but higher in the heart. This differential expression suggests that GPR88 may play a role in the occurrence and development of hypertension, potentially through its influence on abnormal heart contraction and renin secretion, which can increase blood pressure.

In alcohol use disorder, studies with GPR88 knockout mice have demonstrated enhanced seeking and consumption behavior for alcohol, indicating that GPR88 may regulate alcohol consumption [30]. The functional changes of GPR88 in the brain reward system and decision-making processes may affect the reward effect of alcohol and consumption control in individuals with alcohol use disorder.

Regarding anxiety, GPR88 is expressed in brain regions closely linked to anxiety, such as the central amygdala, ventral striatum, and terminal nucleus of the striatum. GPR88 knockout mice have shown decreased anxiety levels, suggesting that GPR88 may play a role in regulating anxiety-like behavior. The mechanism of action for GPR88 in anxiety may involve the modulation of neurotransmitters like dopamine and GABA.

4.4.4. Summary of GPR88. GPR88 is a class C G protein-coupled receptor expressed in various brain regions and transiently in certain peripheral tissues. It has agonists called 2-PCCA and 2-AMPP. GPR88 has been implicated in hypertension, alcohol use disorder, and anxiety. It may play a role in abnormal heart contraction and renin secretion in hypertension. In alcohol use disorder, GPR88 knockout mice show increased alcohol consumption, while its knockout decreases anxiety levels. Further research is needed to explore the mechanisms and develop targeted therapies.

5. Conclusion

The oGPCRs possess a broad spectrum of clinical and medicinal implications. Investigating their native agonists and delineating their signaling pathways across diverse systems holds the potential to revolutionize drug discovery and advancement. Leveraging recent advancements in biotechnology, structural biology, and receptor pharmacology has enabled us to elucidate the ligand-receptor interactions and signaling cascades of certain receptors. However, the current process of de-orphaning is relatively slow, and higher throughput de-orphaning methods need to be developed. In addition, it is difficult to establish a systematic theory of the signaling pathway. In the forthcoming research on oGPCRs, a focal point should be the interplay between receptor functionality and signaling pathways, thus furnishing a theoretical foundation for drug development.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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Preparation scheme and research progress of GPCR antibody drugs

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Abstract. As a class of proteins that play crucial roles in physiological and pathological processes within the human body, G protein-coupled receptors (GPCRs) have emerged as prominent targets for drug development. However, there is still ample room for improvement and replacement of currently developed drugs due to their inherent toxicity and associated side effects. In recent years, the attention has shifted towards antibody-based therapeutics targeting GPCRs, owing to their exceptional specificity and low plasma clearance rate. This review provides an overview of the fundamental preparation process and existing challenges encountered in developing GPCR antibody drugs, along with an introduction to several strategies employed in their formulation.

Keywords: GPCR drugs, antibody, Glutazumab, antigen, erenumab.

1. Introduction

The GPCR is extensively present in various organs and tissues, including the central nervous system, immune system, cardiovascular system, retina, and other body parts. It plays a crucial role in the body's development and maintenance of normal physiological functions. If the intracellular signalling pathway is dysregulated, or if somatic cells are targeted by external pathogenic factors as receptors, a range of diseases can ensue. Scientists from European molecular biology laboratory-European Institute of Bioinformatics and other institutions have classified and summarized the FDA-approved drugs according to known targets. According to statistics, GPCR proteins account for 12% of the drug targets of proteins in the human body; among small molecule drugs, 33% are targeted at GPCR protein family. At the same time, they classified and summarized the approved drugs up to 2015 (without distinguishing small molecule drugs and biological drugs) for the four most popular targets of GPCR, nuclear receptor, ion channel and kinase, and found that the number of drugs developed based on GPCR targets was as many as 370, accounting for the largest proportion; and the ratio of the number of approved drugs in recent 5 years to the total number of approved drugs was also high, showing a rapid growth [1, 2]. The above data show that GPCR, as a class of proteins that play an important role in the physiological and pathological processes of the human body, has always been one of the main targets of drug development. However, there is still room for improvement and replacement of the developed drugs due to toxic side effects and other deficiencies. In recent years, antibody drugs targeting GPCR have begun to enter people's attention. With their excellent targeting and low plasma clearance rate, these drugs have rapidly

won widespread attention in the pharmaceutical field. However, only two GPCR antibody drugs have been listed so far, which proves that there are still great difficulties in the preparation of GPCR antibody drugs. It is very important to find new preparation methods for GPCR antibody drugs.

2. The structure of GPCR and connection with disease

As a crucial protein within the human body, alterations in the structure and functionality of GPCR can impact the metabolic processes and contribute to the development of certain ailments. In the development of antibody drugs targeting GPCR, scientists primarily emphasize studying its extracellular region's structural characteristics. The GPCR consists of seven α -helix segments spanning the cell plasma membrane. The N-terminal and three loops are positioned externally, facilitating interaction between the receptor and its ligand. On the other hand, the C-terminal and three loops are located internally within the cell. Among these, both the C-terminal and third loop play a crucial role in mediating intracellular signal transduction by interacting with downstream G proteins. Consequently, specific ligands binding to GPCR trigger G protein activation, leading to the generation of second messengers like Ca^{2+} or cAMP. These messengers then transmit extracellular signals received by GPCR downstream. However, it is worth noting that apart from relying on G proteins for signal transduction, GPCR can also regulate downstream pathways through interactions with β -arrestin and other molecules without their involvement. The main mechanism of action of GPCR drugs is to use antibodies to specifically bind to different GPCR targets, thereby blocking the extracellular signal that is the activation of the ligand to the target, thereby regulating the cell signal transduction pathway, and then affecting the physiological state and metabolic mode of cells to treat different diseases [3].

Numerous research studies have provided evidence indicating the involvement of several GPCR family members in the initiation and progression of diverse cancer types. Hormone receptor GPCR proteins play a significant role in hormone-dependent cancers, including androgen receptor AR, which facilitates the proliferation of cells in prostate cancer upon activation [4]. Certain receptors, including PAR1, can be activated by MMP-1 and subsequently trigger signalling pathways that facilitate the invasion and progression of cancer cells. Additionally, chemokine receptors like CXCR2 and CXCR4 are frequently overexpressed in myeloma and lymphoma cells, as well as potentially solid tumour cells such as those found in pancreatic cancer. These receptors play a role in both cell migration and angiogenesis [5]. GPCR proteins are involved in many signalling pathways and cell functions, and their roles in various types of cancers are different. Moreover, GPCR is also closely related to inflammation. The chemokine family in the GPCR protein superfamily is mainly expressed on all kinds of cells in the immune system, and participates in the physiological and pathological processes of the development, migration, survival and immune function of immune cells. Among them, inflammation is a kind of pathological phenomenon mediated by the chemokine receptors on immune cells activated by chemokines to mediate the immune cells to exert the host immune function. For instance, neutrophils predominantly express CXCR1 and CXCR2. In the event of infection or injury in specific body regions, neutrophils migrate towards the affected site due to the chemotactic influence of corresponding chemotactic factors (CXCL1, CXCL2, CXCL8). Upon reaching the lesion site, they release inflammatory mediators that induce localized inflammation. In general, there are many types of cancer-related GPCRs with complex functions, but their expression distribution has a certain specificity. By detecting the types of GPCRs highly expressed on the surface of cancer cells, people can develop corresponding antibodies and use them for treatment. The role of GPCR proteins in inflammatory responses is mainly to chemotactic immune cells migrate to the lesion under the action of the corresponding chemokines, such as the chemotactic effect of CXCR1/2 on neutrophils. Developing antibody drugs targeting chemokines or chemokine receptors and blocking the binding of chemokines to their receptors will help reduce inflammatory responses caused by neutrophils.

3. Preparation difficulties and solutions of GPCR antibody drugs

3.1. General preparation and difficulties of GPCR antibody drugs

As an emerging drug, antibody has many characteristics that are not available in traditional small molecule chemical drugs, such as target specificity, very low plasma clearance rate and fewer side effects. Generally speaking, there are two viable approaches for screening humanized therapeutic antibodies. The first method involves genetically modifying the antibody to increase its human component and subsequently selecting hybridoma cells from immunized animals. Alternatively, hybridoma cells can be selected after immunizing transgenic animals with the antigen XenoMouse™ in order to achieve humanization. [6]. Screening methods are divided into in vivo immunization and display screening. In vivo immunization method is to immunize animals with antigen, and then isolate B cells from the spleen of animals, prepare hybridomas, and screen monoclonal antibodies. This method was developed in the 1970s, and is still the main method for pharmaceutical companies to obtain candidate antibodies. The main methods of antibody display are: phage display, yeast display, ribosome display, etc [7]. The advantages of phage display are large library capacity and simple operation, and the disadvantage is that the protein expressed by prokaryotic system lacks the modification of eukaryotic system; the advantage of yeast display is the combination of screening and detection. No matter what antibody screening technology is used, the preparation of pure GPCR antigen with natural conformation in the human body is crucial for the successful screening of humanized anti-GPCR antibodies with therapeutic function. However, only two GPCR antibody drugs are currently on the market, namely Amgen has developed erenumab, a therapy that specifically targets the calcitonin gene-related receptor (CGRPR) to treat migraines [8]. Also, Mogamulizumab, a monoclonal antibody against CC chemokine receptor 4 (CCR4) developed by Kyowa Hakko Kirin [9].

The technical difficulty of GPCR antibody preparation lies in the key step of preparing humanized GPCR antigen with natural conformation, which is due to the following reasons: (1) The purification of GPCR, which consists of 7 transmembrane proteins, poses challenges due to its low expression level in cells. Consequently, obtaining purified samples for subsequent animal experiments and antibody preparation becomes a difficult task. (2) Identifying appropriate targets is a complex endeavor within the structure of GPCR as its extracellular region offers a vast surface area. This makes it challenging to pinpoint suitable targets for different antibodies.

3.2. Preparation scheme of GPCR antibody drug

3.2.1. Using the GPCR extracellular fusion protein as new antigen. In order to solve the problem of the difficulty in purification of large volume and large area GPCR protein antigens, researchers have attempted to use the coupling of the extracellular domain of GPCR protein and carrier protein as a new GPCR antigen, which makes the volume of GPCR antigens smaller and easier to bind to antibodies. The researchers designed a simple method for making a new antigen by chemically coupling a peptide containing the extracellular domain of GPCR to a carrier protein [10].

For example, the conjugation of the bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) to two distinct carrier. In this way, one of the proteins is used for immunization, the other one is used for screening antisera for immunoreactivity [11]. The screening of therapeutic antibodies against GPCR extracellular domains of immunized animals can be accomplished using this new antigen. Although the extracellular structure prepared by this method differs greatly from the conformation of the natural GPCR extracellular structure, the GPCR protein prepared by this method can express some glycosylated GPCR extracellular domain peptides in some cells. [12].

Several GPCR antibody medications have been developed using a similar approach, including erenumab mentioned earlier. Erenumab is an antibody for the treatment of chronic migraine. Its mechanism of action is to regulate the activity of calcitonin receptor-like receptor (CLR), in addition, it can interact with RAMP protein. Researchers found a specific binding site on the extracellular domain of RAMP1, and designed a heterodimer fusion protein consisting of the extracellular domain of CLR

and the extracellular domain of RAMP as an antigen for the preparation of antibodies, thus obtaining Erenumab [13]. The prepared heterodimer Fc fusion was immunized into transgenic mice XenoMouse to produce erenumab, and then hybridoma screening was performed to obtain a large number of therapeutic antibodies. Mogamulizumab is an antibody that has been humanized and specifically designed to target CC chemokine receptor 4 (CCR4), a protein commonly found in various types of blood cancers such as cutaneous T-cell lymphoma (CTCL). Keyhole leukoprotein (KLH) was fused with the N-terminal extracellular domain to isolate anti-CCR4 antibodies, which were then injected into mice for immunization and antibody production [14].

3.2.2. Using cells that express PRCR or Membrane component as antigen. Cells harbouring undamaged and associated proteins that exhibit GPCR or its membrane fragment as an antigen, which encounters challenges in expressing on the cellular membrane owing to diverse impurities present in the membrane. Therefore, this method is currently subject to many limitations. On the other hand, membrane fragments extracted from cells expressing the target GPCR are also used as an antigen. The advantage of using cell fragments and cells with overexpressed proteins as antigens is that the proteins are in their natural conformation. The disadvantage is that the expression level of GPCR protein is very low relative to the total protein expression level on the membrane, and the antibody is more likely to bind to the membrane and other components on the membrane, which may lead to many non-specific results in the screening. Recombinant membrane proteins with accurate tertiary structure are considered optimal antigens for generating monoclonal antibodies targeting membrane proteins. However, cell-based expression systems have limitations such as low expression levels and protein aggregation. To address these challenges effectively, the utilization of double-layer dialysis has shown promising potential in achieving high yields and success rates. Notably, a recent study successfully employed double-layer dialysis to produce 25 different types of GPCR proteins and subsequently screened numerous anti-GPCR antibodies against four specific GPCRs using cell-free synthesis as antigen sources. In addition, this study successfully generated over 40 monoclonal antibodies targeting DRD1 with exceptional affinity and specificity for natural DRD1. These antibodies have broad applications in various research areas, including IHC [15].

3.2.3. Using the whole purified GPCR protein as antigen. Various approaches have been attempted to generate synthetic GPCR proteins that mimic the natural GPCRs' structure. These methods involve using suitable detergents to eliminate impurities from the cell membrane and extract the complete GPCR protein. Although several techniques have proven successful in extracting intact GPCR proteins, their preparation protocols differ depending on the specific type of GPCR. Moreover, it is important to note that purified GPCRs may exhibit structural variations compared to their natural counterparts, potentially affecting their affinity with cellular GPCRs. Nevertheless, utilizing purified whole GPCR proteins as antigens can effectively remove unrelated components present on the cell membrane, thereby enhancing the isolation of target-specific antibodies against GPCRs. Heptares Therapeutics has developed a StaR technology based on amino acid point mutation which introduces a limited number of site-directed mutations into GPCRs. This approach aims to enhance the thermal stability of purified GPCR proteins without compromising their pharmacological properties [16]. Additionally, by employing mutagenesis and other strategies to stabilize specific inactive or active states of GPCRs, it becomes possible to screen for antibodies specifically targeting these activated or inhibited states. Researchers are currently focusing on addressing two key challenges in efficiently producing functional native-like GPCRs: firstly, improving expression levels of these receptors to enhance preparation efficiency; secondly, overcoming issues related to poor stability during purification processes required for subsequent antibody development purposes. In general, this method still has many limitations and is difficult to be directly used in the preparation of GPCR antibody drugs, so more research is needed.

4. Conclusion

Compared to small molecule medications, monoclonal antibodies offer the benefits of precise targeting, reduced side effects, extended presence in the bloodstream, and the ability to utilize various immune functions mediated by leukocytes. These unique characteristics have contributed to their rapid growth in the field of drug development. GPCR plays a vital role in regulating tumour cell behaviour such as growth, movement, proliferation, progression, and metastasis. Despite several small molecule drugs available for targeting GPCR being on the market, only two therapeutic antibody drugs against GPCR have received FDA approval as of September 16th, 2020. One of the main reasons for the slow development of therapeutic antibodies targeting attractive drug targets is the difficulty in preparing functional (natural or natural-like) GPCR antigens. Clearly, due to their complex structure, GPCRs are extremely challenging antigens from which to isolate therapeutic antibodies. However, as noted above, several solutions have been proposed. With the diversification of preparation methods for GPCR antigens, various cutting-edge antibody isolation platforms have emerged. As a class of proteins that play an important role in physiological and pathological processes of human body, GPCR has been one of the main targets of drug development. However, there is still room for improvement and replacement of the developed drugs due to their toxicity and side effects. Among the 370 disease-related GPCR proteins, many have not yet been marketed as effective drugs. With the gradual maturity of the related technologies of monoclonal antibody drugs, coupled with the huge potential of GPCR protein as a target, it is believed that scientific researchers and pharmaceutical companies will invest more energy in the development of monoclonal antibody drugs for GPCR proteins.

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The influence of dietary sugar intake on colorectal cancer

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Abstract. Research Question: This study aims to investigate the consequences of daily sugar intake on human health, particularly focusing on its role in the development of colorectal cancer. The research question explores the intricate mechanisms linking elevated sugar consumption to colorectal cancer and aims to understand the diverse dietary regimens' influence on this association. **Methods:** A comprehensive analysis was conducted to examine the impact of persistent, elevated sugar consumption on the integrity of the intestinal tract and its correlation with colorectal cancer. Various dietary regimens, characterized by different sugar types and quantities, were studied to understand their role in exacerbating the likelihood of developing colorectal cancer. Detailed investigations were carried out to unravel the underlying mechanisms, including insulin resistance, systemic cellular inflammatory responses, and the shift in colonic biological milieu. Inflammatory mediators and specific pro-inflammatory cytokines were analysed in different tissue compartments to comprehend the complex interplay leading to compromised intestinal barrier integrity and increased susceptibility to colorectal cancer. **Conclusion:** Our study reveals compelling evidence linking high-sugar diets to the development of colorectal cancer. Elevated sugar consumption disrupts the integrity of the intestinal tract, primarily through insulin resistance and systemic cellular inflammatory responses. This disruption leads to an upsurge in inflammatory mediators and specific pro-inflammatory cytokines within various tissue compartments. Consequently, the compromised intestinal barrier loses its ability to prevent the infiltration of pathogenic microorganisms, significantly elevating the risk of colorectal cancer. The diverse range of dietary regimens further complicates this association, highlighting the need for comprehensive research in understanding the intricate role of dietary factors in colorectal cancer.

Keywords: Sugar, Inflammation, Insulin Resistance, Colorectal Cancer

1. Introduction

Cancer stands as a formidable adversary, maintaining its position as the leading cause of global mortality [1, 2]. Within this expansive realm of malignancy, colorectal cancer stands out with its commendable early postoperative survival rate and the potential for prevention [3, 4]. While the initial postoperative phase boasts a remarkable 90% survival rate, once the disease progresses to its middle and late stages, the grim statistics tell a different story, with recurrence and mortality rates soaring beyond 50%. Thus, the importance of vigilant attention and proactive measures in addressing colorectal cancer cannot be overstated. Over the past six decades, Norway has witnessed a notable surge in the incidence of colorectal cancer. Among Norwegian women, the incidence rate has surged from a modest 9.9 cases per

100,000 in the years 1955-1959 to a concerning 52.5 cases per 100,000 during the period spanning 2011-2015. This alarming increase finds a significant correlation with the prevalence of high lactose diets among the Norwegian population [5, 6]. Notably, factors such as diet-induced obesity, which is exacerbated by these high-lactose dietary practices, have been identified as significant contributors to the onset of colorectal cancer [7]. High sugar diets present another facet of dietary influence, as they have been linked to the development of insulin resistance, consequently leading to elevated levels of insulin and insulin-like growth factors. These hormonal shifts potentially serve as catalysts in the development of colorectal cancer [8]. The energetic demands of cancer cells, necessitating a substantial supply of energy, intertwine intimately with our daily sugar intake. In the context of populations with high-sugar diets, the escalation in the number of colorectal cancer cases and incidence rates has prompted a growing body of research indicating that dietary sugar plays a direct role in amplifying the occurrence of colorectal tumors. A pivotal report from the Nurses' Health Study in the United States, conducted over the span of three decades (1984-2014), shed light on this matter. Among the 2,733 confirmed colorectal cancer patients who participated, a disheartening 901 individuals succumbed to colorectal cancer. In this cohort, a discernible and statistically significant positive correlation emerged between dietary sugar intake and both colorectal cancer mortality and incidence. This finding underscores the importance of understanding the intricate interplay between dietary factors, especially sugar consumption, and the development of colorectal cancer [9, 10].

2. Classification of Sugars

In the realm of dietary sugars, a fundamental classification emerges, encompassing three primary categories: monosaccharides, disaccharides, and polysaccharides. These categories offer critical insights into how these sugars interact with the human body, particularly with regard to their digestion and absorption.

Glucose, a monosaccharide, holds a unique status in the world of dietary sugars. It stands as a sugar that the human body can promptly and directly absorb, making it readily available for cellular energy production [11]. In contrast, disaccharides such as sucrose and lactose present a more intricate journey within the digestive system, requiring enzymatic breakdown before their constituent monosaccharides, fructose, and glucose, can be effectively absorbed and utilized.

Sucrose, a prominent disaccharide, constitutes a combination of fructose and glucose. Importantly, the digestion, absorption, and metabolic pathways for these two sugar components diverge notably from one another. This divergence in metabolic fates introduces a layer of complexity when considering the physiological responses to various dietary sugars [12].

Given the diverse dietary practices across populations, it is crucial to recognize that distinct types of sugars are consumed in varying proportions. These dietary variations in sugar types have the potential to exert differing effects on the human body, which necessitates in-depth investigation. To comprehensively grasp the implications of these variations, further research is indispensable in elucidating how different sugar types may contribute to the complex interplay between diet and colorectal cancer. By investigating the intricate relationship between sugar classification and colorectal cancer risk, we can unravel essential insights that may inform preventive strategies and dietary recommendations [13].

2.1. Total Sugar Intake

The intricate relationship between total sugar intake and cancer risk has been a subject of extensive investigation. A notable contribution to this field comes from a meta-analysis conducted by Debras and colleagues, which analyzed data from the NutriNet-Santé cohort spanning the years 2009 to 2019. Their research scrutinized the association between total sugar intake, various categories of added sugar, and dietary sources of sugar with the risk of cancer. This comprehensive analysis unearthed compelling findings. It revealed that moderate elevations in total sugar intake were indeed linked to an overall escalation in cancer risk. However, the magnitude of this risk varied based on whether the increased sugar intake was derived from total sugar or added sugar alone. Specifically, heightened total sugar

intake, as opposed to an exclusive focus on added sugar, was found to be modestly associated with an elevated overall risk of cancer (Hazard Ratio (HR) for Q4 compared to Q1: 1.17; 95% Confidence Interval (CI): 1.00, 1.37). This nuance underscores the importance of distinguishing between the sources of sugar when evaluating their impact on cancer risk [14].

The impact of sugar intake, particularly concerning its association with colorectal cancer, is of significant interest. Colorectal cancer incidence varies notably among populations characterized by high sugar consumption in their daily diets, and this variability is often shaped by ethnic factors. Epidemiological research consistently underscores that increased consumption of whole-grain foods, fruits, vegetables, and dairy products is frequently linked to a reduced risk of developing colorectal cancer. Conversely, high consumption of other fruits and added sugars among African Americans has been strongly correlated with a substantial increase in their susceptibility to colorectal cancer.

Conversely, the dietary patterns of white Americans, characterized by the inclusion of vegetables, fish, poultry, fruit, and whole-grain dairy products, demonstrate a significant reduction in the risk of developing colorectal cancer. It is evident that dietary disparities influenced by cultural and geographical factors are pivotal in shaping the profiles of colorectal cancer risk among different populations.

Moreover, the influence of sugar intake on colorectal cancer risk is not confined to a singular population, as exemplified by a study in Japan. In this research, a cohort of participants comprising 42,405 men and 48,600 women, aged between 45 and 74 years, was followed over a substantial period. Data gathered through a prospective study initiated by the Japan Public Health Center from 1995 to 1999 unveiled intriguing insights. For women, a notable positive correlation emerged between total sugar intake and the risk of colorectal cancer (Risk Ratio (RR) 1.75 [95% CI: 1.07–2.87] for Q1 vs. Q5; p for linear trend = 0.03). However, in the case of men, no statistically significant trend was observed. This gender-specific divergence raises the possibility that even in middle-aged Japanese adults, an underlying increase in colorectal cancer risk associated with higher total sugar intake cannot be categorically dismissed.

In summary, the intricate relationship between sugar consumption and colorectal cancer risk is multifaceted and influenced by numerous factors, encompassing the source of sugar, dietary patterns, and even gender disparities. These complexities underscore the necessity for additional research to refine our comprehension of the intricate interplay between sugar intake and the development of colorectal cancer. Such insights can ultimately pave the way for more precise recommendations pertaining to cancer prevention and dietary strategies [15].

2.2. *Impact of Monosaccharides*

Recent investigations into the landscape of early-onset colorectal cancer have unveiled an unsettling trend intimately tied to the dietary consumption of sugar, particularly focusing on the role of monosaccharides. These studies have delved into the nuanced connection between the ingestion of these simple sugars in early dietary habits and the subsequent development of colorectal cancer.

Within the domain of prospective clinical trials, a significant revelation emerged when examining patients with stage III colon cancer. The findings underscored that an elevated intake of monosaccharides was undeniably linked to a heightened risk of cancer recurrence and mortality. This insight suggests that the repercussions of sugar consumption extend beyond the realm of cancer development, reaching into the vital arena of cancer management, where aspects such as recurrence and survival assume paramount significance.

Expanding on these insights, a prospective cohort study targeting individuals with colon cancer brought forth compelling evidence. It elucidated the detrimental effects of consuming two or more servings of sugar-sweetened beverages on a daily basis. This level of consumption was associated with a substantial 75% increase in the risk of cancer recurrence and a pronounced escalation in the risk of mortality when compared to those who maintained a more conservative sugar-sweetened beverage intake (95% Confidence Interval: 1.04–2.68). This observation underscores the pivotal role of monosaccharides, particularly when delivered in the form of sugar-sweetened beverages, in influencing

the trajectory of colorectal cancer. This influence is discernible in terms of both disease recurrence and patient survival.

In the context of early-onset colorectal cancer, these findings paint a vivid picture of the substantial impact of monosaccharide intake on disease outcomes. This places the spotlight firmly on the need for a comprehensive understanding of the role of sugar, especially in its simplest form, in colorectal cancer etiology and progression. Further research and in-depth exploration of these connections are imperative to guide interventions that can effectively mitigate the risks and consequences associated with the consumption of monosaccharides in the context of colorectal cancer [16].

2.3. *Impact of Disaccharides*

The intricate relationship between sugar intake and its influence on colorectal cancer risk delves further into the specific role of disaccharides, uncovering pronounced associations with sucrose, non-fruit-derived sugars, and added sugars. Among the array of disaccharides, lactose, composed of glucose and galactose, stands out alongside lactulose (comprising fructose and galactose) and lactitol (galactosyl-sorbitol), each potentially yielding similar effects within the lower small intestine. Underpinning the link between disaccharides and colorectal cancer risk are potential biological mechanisms that bear investigation. Notably, higher intake of disaccharides has been correlated with increased insulin secretion and heightened inflammatory responses. These physiological responses are considered integral in the path toward the development of colorectal cancer. An additional dimension to this narrative is the pertinence of circulating fasting insulin levels. Research reveals that elevated levels of fasting insulin are instrumental in elevating the risk of colorectal cancer, with a notable emphasis on its impact on male colorectal cancer risk concerning the rise in glycated hemoglobin concentration [7].

These discoveries serve as a catalyst for exploring potential preventative strategies in the context of colorectal cancer. Recent research suggests that interventions, whether they be pharmaceutical or lifestyle-oriented, directed at lowering circulating insulin levels, may hold promise in the prevention of colorectal tumors. This emphasizes the potential utility of insulin as a modifiable factor in reducing the risk of colorectal cancer. The influence of disaccharides extends far beyond the cellular level and reaches into the intricate ecosystem of the colorectal microbiota.

Alterations within this microbial community have been observed in response to disaccharide consumption, while the overall equilibrium between aerobic and anaerobic bacteria remains largely unaffected [17, 18]. Notably, in germ-free rats, the effects induced by lactulose are absent, signifying the pivotal role of the microbiota in mediating the connection between disaccharides and colorectal cancer risk. The therapeutic potential of this microbial influence has also been explored, with the administration of encapsulated lactobacillus strains to individuals with hepatic encephalopathy demonstrating therapeutic effects. This further substantiates the intricate interplay between disaccharides, the microbiota, and specific disease outcomes.

Within this framework, it becomes increasingly evident that disaccharides hold significant relevance in the etiology and progression of colorectal cancer. Nevertheless, the intricacies of this relationship underscore the imperative for comprehensive research to unveil the mechanisms underpinning the role of disaccharides in colorectal cancer. In summary, a concise overview of a prospective cohort research indicates that the consumption of sugar-sweetened carbonated beverages does not yield a statistically significant increase in the risk of colorectal cancer (95% Confidence Interval: 0.66–1.32). This highlights the critical need for further investigation and a more profound comprehension of the mechanisms through which disaccharides exert their influence on colorectal cancer [19].

3. Results

3.1. *Insulin Resistance in CRC*

The implications of sugar-induced insulin resistance in the context of colorectal cancer are multifaceted, as underscored by the research conducted by Hamid Farahani in Norway. This study delved into the plasma levels of insulin and resistance, providing critical insights into their role in colorectal cancer

(CRC). Notably, the Homeostatic Model Assessment of insulin resistance revealed a substantial disparity between CRC cases and the control group, with values significantly higher in CRC cases (1.8 ± 0.4) compared to the control group (1.4 ± 0.3), emphasizing the relevance of insulin resistance in the disease's pathophysiology ($P < 0.001$). Furthermore, when scrutinizing CRC cases by tumor location, the findings became even more compelling. Higher levels of resistance and insulin were observed in colorectal cancer cases, with HOMA-IR significantly surpassing that of the control group (resistance: 5.9 ± 1.2 vs. 5.4 ± 1.3 , $P = 0.043$; insulin: 5.9 ± 1.2 vs. 5.4 ± 1.3 , $P = 0.039$; HOMA-IR: 1.9 ± 0.4 vs. 1.3 ± 0.3 , $P < 0.001$). This nuanced stratification further elucidated the role of insulin resistance in different anatomical contexts within the colorectal region, underscoring its significance in CRC pathogenesis. An intriguing facet of this study was the observed positive correlation between insulin resistance and insulin levels, a relationship found in both the control group ($r = 0.737$, $P < 0.001$) and colorectal cancer cases ($r = 0.881$, $P < 0.001$). These correlations suggest the intricate interplay between insulin and resistance and their shared influence in colorectal cancer [20].

The epidemiological context of colorectal cancer reveals notable trends, particularly evident in the case of Finland, where the incidence of colorectal cancer has more than doubled since the 1950s. This increase has led to a significant rise in cases, with dietary and modifiable factors contributing to as much as 90% of these instances [21]. Of particular interest is the role of high insulin levels, which have been associated with the activation of insulin-like growth factor 1 (IGF-1) receptors, thereby enhancing the potential for carcinogenesis by inhibiting apoptosis [22]. Laura A. Colangelo's extensive research extended this investigation, utilizing data derived from a survey encompassing 191,126 men and 20,433 women, excluding diabetic patients. Among this cohort, there were 433 recorded cases of colorectal cancer-related deaths among men and 149 cases among women.

The examination of risk factors linked to insulin resistance syndrome yielded a relative risk (RR) that presented compelling evidence. Men with at least three of the four risk factors - being in the PLG gender-specific highest quartile, having elevated systolic blood pressure, a high body mass index, or an increased resting heart rate - exhibited a noteworthy RR of 1.67 (95% Confidence Intervals (CI): 1.04-2.70) compared to those not in the upper quartile for these factors. In the case of women, the RR was 1.29 (95% CI: 0.70-2.37). Importantly, this association held consistent for both men and women, with a RR of 1.50 (95% CI: 1.03-2.19). These findings underline the existence of a moderate connection between PLG and insulin resistance syndrome and colorectal cancer mortality, offering substantial support for the insulin hypothesis.

In conclusion, the complex interplay between insulin resistance, sugar-induced factors, and colorectal cancer unveils a multifaceted landscape. The significance of these findings prompts further exploration to understand the intricate mechanisms underpinning the role of insulin resistance in colorectal cancer etiology and progression [23].

3.2. *The Impact of Inflammation on CRC*

The intricate relationship between inflammation and colorectal cancer (CRC) unfolds as a pivotal determinant in both disease onset and survival [24]. The interconnectedness of local and systemic inflammatory responses emerges as an influential and independent predictor of colorectal cancer and its ultimate prognosis. Diet plays a pivotal role in shaping this intricate relationship. Different diets have the power to significantly alter the composition of the gut microbiome. Notably, excessive consumption of sucrose appears to exert a more profound impact on metabolic changes, while fructose takes center stage in inducing intestinal barrier dysfunction and inciting subclinical inflammation. Interestingly, the absorption of sucrose serves to enhance the bioavailability of fructose, leading to metabolic outcomes like obesity and potentially sugar addiction. The increasing intake of various sugar types contributes to the development of chronic inflammation within the intestinal environment [25].

In a comprehensive report by Sergei I. Grivennikov, the multifaceted roles of immune cells, cytokines, and other immune mediators come to the fore, illustrating their involvement in nearly all stages of colorectal tumor development, spanning tumorigenesis, promotion, progression, and even metastasis [26]. For an extended period, a high sugar intake has been identified as carrying a notable risk for

inflammatory bowel disease, marking a critical connection between dietary sugars and intestinal inflammation [27].

Excessive sugar consumption exerts a deleterious impact by disrupting metabolic processes, giving rise to an increase in inflammatory mediators and specific pro-inflammatory cytokines across various tissues. This disruption sets the stage for the emergence of low-grade chronic inflammation. In parallel, elevated fructose intake exacerbates this scenario, as it heightens intestinal permeability, facilitating the release of inflammatory factors into the liver. This, in turn, triggers elevated levels of liver and systemic inflammation [28, 29].

A crucial consequence of high sugar intake is the reduction in microbial diversity within the gut microbiome. This dietary effect leads to a depletion of short-chain fatty acids (SCFAs) within the intestinal lumen. SCFAs play a pivotal role in shaping the recruitment of regulatory T cells and influencing the antimicrobial activity of macrophages, both of which contribute to the overall functionality of the mucosal immune system [30].

Central to this intricate cascade of events is the integrity of the intestinal barrier. When the intestinal barrier becomes compromised, its ability to ward off the invasion of pathogenic microorganisms is compromised. This breach paves the way for the translocation of substances like lipopolysaccharides (LPS) originating from *Escherichia coli*. These LPS and analogous molecules activate specific receptors, such as Toll-like receptor 4 (TLR4), thereby triggering downstream Nuclear factor-kappa B signaling pathways. The outcome is the induction of heightened levels of inflammatory factors, including IL-1 β , TNF- α , and IL-6, accompanied by an increase in neutrophil infiltration. These collective events contribute to the potentiation of colorectal cancer, with more severe manifestations stemming from this chain of inflammatory events [31].

In summary, the intricate interplay between sugar consumption, inflammation, and colorectal cancer is an area of paramount importance. These multifaceted connections demand in-depth research and investigation to illuminate the underlying mechanisms. Understanding this interplay is vital in the context of developing preventive strategies and interventions that can effectively curb the impact of sugar-induced inflammation on colorectal cancer [32].

3.3. *The Impact of BMI on CRC*

Amidst global epidemics of obesity and cardiovascular diseases, the issue of high dietary sugar intake emerges as a prominent concern, further compounding the perils of excessive sugar consumption. This concern is underscored by the fact that, according to the 2005 U.S. Dietary Guidelines, the consumption of added sugars significantly surpasses recommended limits for discretionary calorie intake, irrespective of energy requirements. A stark illustration of this is seen in the period between 2001 and 2004, when the average daily intake of added sugars in the United States reached a staggering 22.2 teaspoons per day, equating to 355 additional calories daily. This marked increase is part of a more substantial trend that unfolded from 1970 to 2005, during which the average annual availability of sugars and added sugars surged by 19%, contributing an extra 76 calories to the daily energy intake of the average American [33].

Amidst these shifts in dietary patterns, the association between Body Mass Index (BMI) and sugar-induced obesity takes on significant importance, given its profound implications for the risk of colorectal cancer development. An extensive study conducted by the Department of Surgery at Erlangen University Hospital meticulously examined the survival rates of 612 patients diagnosed and treated for colorectal cancer between 2003 and 2010. The findings are quite revealing, as they indicate that patients classified as having obesity class II or higher (BMI ≥ 35 kg/m², n = 25) and those falling into the underweight category (BMI < 18.5 kg/m², n = 5) experienced reduced overall survival (hazard ratio (HR) = 1.6; 95% confidence interval (CI) 0.9–2.7) and a higher incidence of distant metastases (HR = 1.7; 95% CI 0.9–3.3) in contrast to patients with normal body weight (BMI ranging from 18.5 to < 25 kg/m², n = 209), those classified as overweight (BMI ranging from 25 to < 30 kg/m², n = 257), or individuals in obesity class I (BMI ranging from 30 to < 35 kg/m², n = 102).

Notably, it's essential to emphasize that both underweight and overweight conditions were linked to decreased overall survival and elevated rates of distant metastasis among individuals diagnosed with colorectal cancer [34].

Moreover, gender disparities have a significant effect on the intricate relationship between Body Mass Index (BMI) and colorectal cancer. Data indicates that obese men exhibit a heightened risk of developing colorectal cancer in comparison to men with normal body weight. However, this association is less pronounced in women, with no significant correlation observed in obese women. This gender-specific variation in risk factors underscores the complexity of the interactions between BMI and colorectal cancer among different gender cohorts [35].

Epidemiological evidence consistently points to obesity being linked to a 30% to 70% increase in the risk of colorectal cancer in men, although this association is less uniform in women. Similar trends can be observed concerning colorectal adenomas, albeit with a less pronounced risk profile. It's worth noting that visceral fat, or abdominal obesity, appears to be of more significant concern than subcutaneous fat obesity. A mere 1 kg/m² increase in BMI is associated with additional risk (hazard ratio 1.03). This manifestation underscores the importance of considering not only overall BMI but also the distribution of adipose tissue.

Importantly, it should be acknowledged that obesity may have consequences that extend beyond colorectal cancer risk. Indeed, obesity might be correlated with unfavorable cancer outcomes, as evidenced by higher rates of primary cancer recurrence and increased mortality. The intricate interplay of dietary sugar intake, BMI, and colorectal cancer risk underscores the necessity for comprehensive research endeavors to elucidate the underlying mechanisms. These insights are pivotal for the development of precisely targeted interventions and preventive strategies aimed at mitigating the multifaceted impacts of sugar-induced obesity on colorectal cancer.

4. Conclusion

In conclusion, this review reveals a discernible correlation between sugar consumption and the risk of colorectal cancer (CRC). Among various sugar types, sucrose, non-fruit-derived sugars, and added sugars stand out as having a more pronounced impact on CRC. It is worth noting that the current body of evidence linking sugar intake to CRC primarily relies on findings from case-control studies. The extent to which different populations in diverse geographic regions are influenced by dietary sugar intake exhibits variation. High-sugar diets appear to have a comparatively smaller impact on Japanese individuals, while they exert a more substantial effect on African Americans, closely linked to their dietary patterns.

This paper takes into consideration the influence of multiple factors such as obesity, high salt intake, sleep patterns, among others, in an effort to establish a connection between CRC and dietary sugar habits. The primary aim is to underscore the robust positive association between excessive sugar-rich diets and the occurrence of CRC. However, it is important to acknowledge that this association demonstrates subtle variations across different regions and among diverse ethnic groups due to variations in dietary cultures. In CRC cases attributed to high-sugar diets, factors like insulin resistance and systemic inflammatory responses are likely to play pivotal roles. Nevertheless, further experimental data is essential to conclusively establish the relationship between daily sugar intake in humans and CRC.

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CRISPR/Cas9 in the treatment of β -thalassemia

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Abstract. Based on clinical evidence, medication resistance poses a significant challenge to the treatment of cancer. It causes the disease to become uncontrollable and raises death rates. Drug resistance arises from a variety of causes, but a change in the inherited makeup of tumor cells is typically the root reason. The ability to modify the genome is growing with the recent discovery of clustered regularly interspaced short palindromic repeats (CRISPR)/associated (Cas)9 technology, which may be helpful in reducing drug resistance. Owing to its exceptional accuracy and efficiency, the CRISPR/Cas 9 system has been used to investigate the relevant roles of cancer-causing genes, create animal models of tumors, and identify potential therapeutic targets. As a result, it has emerged as the go-to technique for therapeutic gene editing. Utilizing CRISPR/Cas 9 technologies in the treatment of different diseases is growing. Because oncogene regulation differs from normal gene regulation, the CRISPR/Cas 9 system offers efficient methods for oncogene elimination, interference with expression, and modification of activity, all of which can effectively impede the growth of tumors. This article discusses the potential of the CRISPR/Cas9 system to identify resistance targets in drug-resistant breast cancer and reverse resistance gene alterations. Furthermore, the difficulties that prevent this technology from being clinically applicable and emphasize the CRISPR/Cas9 systems are discussed. The CRISPR/Cas9 system will be a crucial component of personalized medicine and is anticipated to have a significant impact on reducing drug resistance in cancer therapy.

Keywords: CRISPR/Cas9, medical applications, cancer.

1. Introduction

The illness known as cancer arises from an imbalance in normal regulation and an overabundance of cell division in the body. Cancer cells are defined as hyperproliferating cells that frequently infect nearby tissues and even spread to other parts of the body through the lymphatic and/or circulatory systems (cancer metastasis). Notably, malignant tumors include cancers and sarcomas. A cancer is a malignant tumor, not a cancer. There are numerous varieties of cancer, and the location, malignancy, and metastasis of the cancer all affect how severe the disease is. Once diagnosed, it is often treated by combining surgery, chemotherapy and radiotherapy. If the cancer is untreated, the final result usually results in death. With the ubiquity of bad lifestyles, cancer is becoming more and more frequent. According to the International Center for Cancer Research (International Agency for Research on Cancer, IARC) A Cancer Journal for Clinicians Global Cancer Statistics Report 2018: Estimates of the incidence and Mortality of 36 cancers in 185 countries worldwide ① shows that there were 1,8078,957 new cancer cases worldwide in 2018, The incidence rate is 236.9/10 ten thousand; 9 555 027 cancer deaths, Mortality rate is 125.2/10 ten

thousand. Among them, new cancer cases and deaths in China accounted for 23.7% and 30% of ②, respectively. That means that there were 4,285,033 new cancers in China in 2018, of which 2,865,174 cancer patients died [1].

The International Institute of Cancer has released its 2020 Global Cancer Data, which shows that breast cancer is now the most common cancer worldwide in women, surpassing lung cancer in terms of new cases. In China, the growth rate of breast cancer incidence of cancer is twice that of the global growth rate, ranking the top in the incidence of malignant tumors in the female population. This leads to an increasing number of patients and medical stress. As the most widely distributed cancer among Chinese women, breast cancer is characterized by high survival rate and long treatment time. This leads to an increasing number of patients and medical stress [2]. Patients were aged 26 - 73 years, The mean age was 51.91 ± 9.47 years, Most of the patients were aged 45 - 59 years, For middle-aged women, 91.98% of the patients were married with a living spouse; More than half of the patients were not highly educated, Junior high school (37.79%) and primary school and below (34.73%); retired patients (28.63%) and unemployed patients (27.48%) Nearly employed patients could not work during chemotherapy (33.59%); nearly half of them had a monthly income of 1000 - 3000 (43.51%); most of the patients had urban medical insurance (68.32%) [3].

There are three phases to the CRISPR/Cas system's operation. The acquisition of additional spacers is the first step, CRISPR locus expression, including transcriptional and post-transcriptional processing, is the second, and disruption of the CRISPR/Cas system is the third [4]. Recent research on gene function, virus-host interactions, and recombinant vaccines has benefited greatly from the use of CRISPR/Cas 9. Its high efficiency, simple design and wide application provide strong support for these studies. In many cases of viral genome editing, CRISPR/Cas 9 has shown higher editing efficiency by simultaneously editing multiple sites in the viral genome to produce a multivalent recombinant vaccine against highly infectious strains. The development of recombinant vectors / plasmids with multiple antigens will be important for vaccine development, especially in the context of outbreaks of coronavirus and its varieties, and the importance of future vaccine development is more prominent. Therefore, the development process of multivalent vaccines should focus on a broad host range and rapid development [5].

2. CRISPR technology

The CRISPR system was initially identified in 1987. But it hasn't been widely applied until 2013 that CRISPR technologies began to be widely used. Over the last five years, CRISPR has revolutionized not only the domains of chemical biology and medicine, but also biotechnology and biotechnology. Following the initial successful use of these technologies in the genetic alteration of mammals, experts from all over the world contributed favorably to the advancement of these technologies using their specialized knowledge.

Widespread in bacteria and archaea, CRISPR systems are adaptable immune systems. Dr. Nakata's team identified a unique repeat sequence in *Escherichia coli* (*E. coli*) in 1987 [6]. Two decades later, in 2002, the sequence was dubbed CRISPR, or arranged in a regular, spaced-out short palindromic repeat. Simultaneously, the genes next to the repetitive sequences were discovered to be CRISPR-associated (Cas) genes. The fundamental elements required for the process of adaptive immunity are the CRISPR array and Cas proteins. Since 2007, more light has been shed on the following unraveling of the molecular intricacies of CRISPR-Cas systems. In vitro cleavage of target DNA by pure Cas9 under the supervision of crRNA/tracrRNA was demonstrated in 2012. Later in 2013, mammalian cells were able to change their genomes using the type II CRISPR Cas9 technology. Subsequently, several CRISPR-Cas single effector enzymes have been discovered and altered for use in a variety of species.

CRISPR-Cas systems are split into two classes, Class 1 and Class 2, based on notable differences in the Cas proteins involved in binding guidance and target cleavage. These classes are further grouped into six groups based on the "signature genes." Based on their multi-subunit effector complexes, Type I, III, and IV systems are classified as Class 1 systems; on the other hand, Type II, V, and VI systems' single, massive, multi-domain effectors are categorized as Class 2. The researchers are still looking into different CRISPR systems, therefore the classification work is not yet complete. Furthermore, the

classification has to be updated as new information based on structural, biochemical investigations, and comparative genomic research of CRISPR components becomes available. The review by Koonin et al. provided an overview of the most recent categorization of CRISPR-Cas systems. The Type II system, which had Cas9 from *Streptococcus pyogenes*, and the Type V system, which contained Cas12a (formerly known as Cpf1) from *Acidaminococcus* sp (AsCas12a), the *Lachnospiraceae* bacterium (LbCas12a), and *Francisella novicida* (FnCas12a), were the most commonly utilized systems (figure 1) [7, 8].

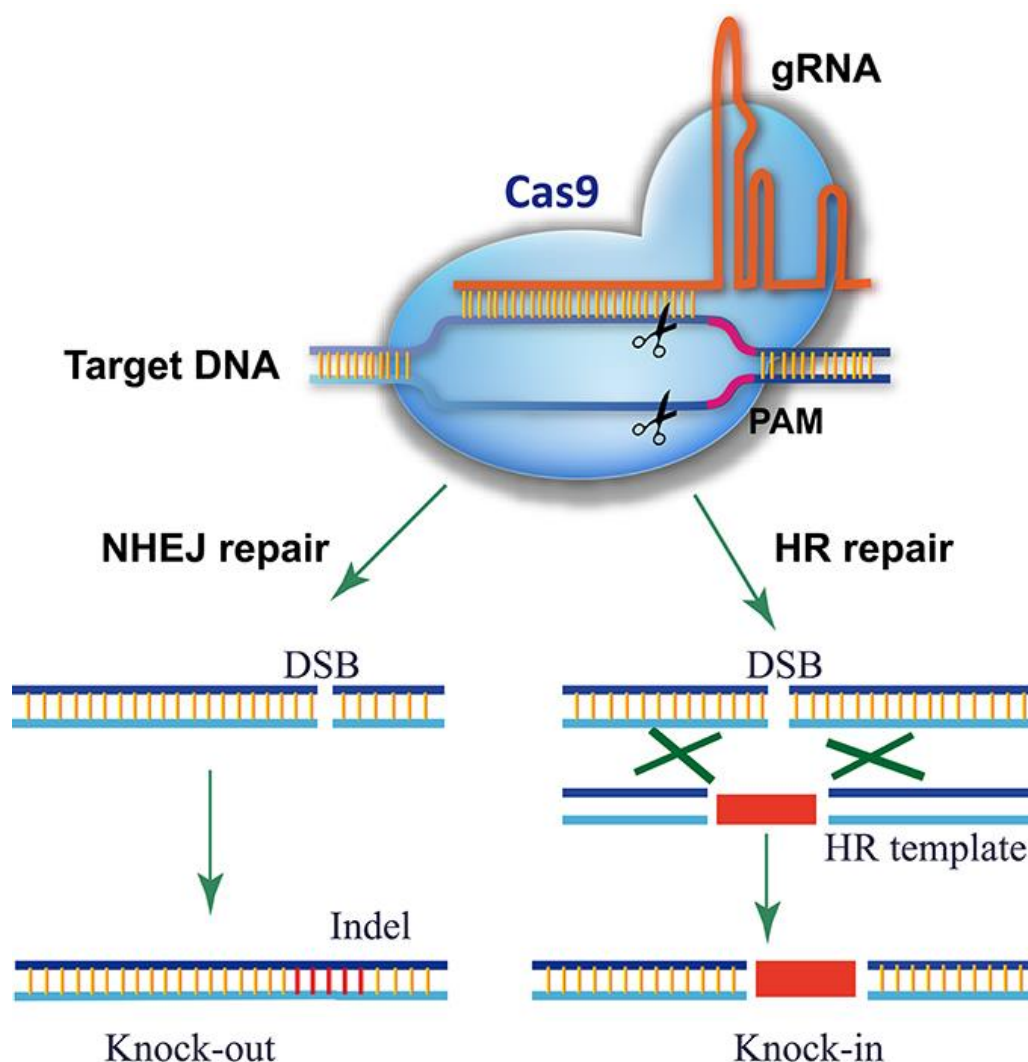


Figure 1. Schematic of Cas9/gRNA genome editing [9].

3. CRISPR/Cas9 Is Better Than Other Methods for Overcoming Drug Resistance in Breast Cancer.

Traditionally, medicines with distinct targets have been combined to reduce acquired drug resistance. But because one drug's methods of action interact with the others, it is difficult to predict the outcome. Enhancing the anticancer agent's specificity is a further way to reduce the likelihood of acquired drug resistance, particularly in MDR situations. Furthermore, it would be possible to reuse currently available anticancer medications by inhibiting or reversing resistance mechanisms [10].

A sequence of genetic events leads to the multistep and complex process of cancer development, including breast cancer. Numerous point mutations, copy number changes, and chromosome

rearrangements have been found in the genome sequencing of several cancer types. Oncogenes and drug-resistant genes have been validated using a variety of in vivo and in vitro methods. These techniques can be divided into two categories: gain-of-function (cDNA-based over-expression) and loss-of-function (RNAi, for example) of the target gene. In the past few decades, these methods have played a crucial role in many important discoveries in cancer biology, but they also have some serious drawbacks. CDNA based expression systems may elevate gene expression to a supraphysiological level, which may have adverse effects on biological processes within cells. Furthermore, RNA interference (RNAi) only partially knocks down a gene's mRNA levels; residual mRNAs may still be functional. This may make it difficult to identify targets that need whole mRNA inactivation. Although genome engineering in mouse or human cell models is more comprehensive, conventional methods have found it to be time-consuming and technically difficult. The predominant technique for simulating various cancer forms in mice has been the Cre/LoxP system. The intricate genetic processes involved in cancer has made it difficult to use functionally analyze the importance of individual mutations or the combinatorial impact on genes related to carcinogenesis.[11].

The CRISPR/Cas9 technology provides a quick mechanism for specific endogenous locus alteration, circumventing the drawbacks of the previously stated techniques. This approach could be applied, for instance, to somatic genome engineering, genetic screening in vivo or in vitro, CRISPR-based effector regulation, and genetic variant modeling. Recent studies have shown that CRISPR/Cas9-mediated NHEJ or HDR can effectively disrupt or modify genes in different types of cancer cells. It has been effectively applied to patient-derived xenografts and established cell lines to modify, reorganize, or introduce new mutations at several locations in the mammalian genome. Since CRISPR/Cas9-based genome editing has advanced, many of the difficulties in producing.

4. Conclusions

The area of molecular biology has undergone a dramatic shift in the past few years due to the astounding advancements made in the development of numerous CRISPR-based technologies, especially in human cells and mammalian model systems. In addition to the widely utilized Cas9 system, other CRISPR-related nuclease variations are still being identified and explored, offering possible enhancements in accuracy, effectiveness, and transport. Meanwhile, there are countless opportunities for diverse applications with additional improvement of the well-characterized CRISPR systems that are already available. However, there are still certain technical obstacles to overcome. CRISPR-based technologies have enormous Possibility of therapeutic use as a generally applicable method. In June 2016, the United States approved the first CRISPR clinical trial. At the West China Hospital in Chengdu, the first patient received the modified CAR-T cells later in October of that same year. Following the successful correction in 2017 of a detrimental mutation in genes in human embryos, more CRISPR-related clinical trials are being looked at.

But as the CRISPR revolution gains momentum, worries about their application on a social and ethical level are also growing. Greater thought should go into the therapeutic uses of these effective instruments. In May 2018, the CRISPR gene therapy experiment was put on hold by the US Food and Drug Administration (FDA) without providing any information. Undoubtedly, CRISPR-based treatment is still in its infancy and has a number of issues that need to be resolved. The off-target effect is one of the main worries. Various Cas nucleases have been designed to increase the accuracy of targeting. The delivery efficiency of CRISPR-based gene therapy is another significant barrier. In addition to technological obstacles, the public is also interested in social and ethical issues. Although a study suggested that Cas9 might cause humans' adaptive immune reactions, no workable remedy has yet been discovered. In addition, a different investigation noted that Cas9 editing caused unintentional alterations to the human germ line and that it activated a p53-mediated DNA damage response, a characteristic shared by cancer cells. There is still more work to be done in developing CRISPR-based therapy because the reasons for these issues are still unclear. All things considered, we think that when CRISPR is used more and more, amazing advancements and uses may become possible.

Author contributions

All the authors contributed equally and their names were listed in alphabetical order.

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Achievements of artificial intelligence aided diagnosis of breast cancer

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Abstract. Breast cancer has become a major disease threatening women's safety, making people suffer with its high mortality and unbearable pain. The struggle between humans and breast cancer has a history of thousands of years. Even today, with the development of science and technology, breast cancer is still a headache for everyone. The examination and treatment of breast cancer has caused a great medical burden, especially the expensive equipment and examination costs, as well as the test of doctors' professionalism, which makes the poor areas even have insufficient resources to treat. But this struggle has entered a new stage, with the continuous maturity of artificial intelligence and machine learning technology, and the application of artificial intelligence in the medical field, perhaps it can change the current situation. AI can greatly reduce medical costs, and the diagnostic efficiency is much faster than that of doctors. Thanks to AI's low cost and efficiency, the prevention of breast cancer will become easier in the future. This article will mainly introduce AI-assisted diagnosis's achievements in treating and preventing breast cancer in the past four years.

Keywords: Artificial intelligence, breast cancer, medical imaging.

1. Introduction

With the continuous development of technology, people are paying more and more attention to their physical health. From the current medical level, people can completely cure most diseases, but there are still some serious diseases that scientists have not found very effective ways to overcome, such as cancer. Cancer is divided into many kinds, breast cancer is one of them. Before 2020, lung cancer mortality rate is the highest among all cancers. After 2020, breast cancer surpassed lung cancer and became the second highest mortality cancer [1]. Breast cancer accounted for 11.7% of new cases and 6.9% of new deaths [2].

But people are not at a loss when facing cancer. During the long struggle against cancer, scientists and doctors have found that early intervention can slow down the rate of cancer onset and reduce its lethality. Breast cancer is no exception. If we can find signs of breast cancer early and give timely intervention, we can achieve significant results in treating breast cancer. Therefore, early detection and intervention in cancer have become crucial in treating cancer, and determining the ultimate therapeutic effect. Early intervention can effectively reduce the mortality and incidence rate and improve the cure rate [1].

Therefore, how to find the existence of breast cancer early and efficiently and save manpower has become one of the efforts of scientists. Nowadays, there are many mature medical image detection technologies available. Breast cancer can be detected by these imaging techniques: X-ray, ultrasound, and MRI.

X-ray can detect small tumors, so it is more suitable for detecting breast cancer for women who do not feel obvious discomfort [3]. X-ray is a very effective detection technology to reduce breast cancer mortality, even if it will lead to a large proportion of overdiagnosis [1]. Ultrasound is also often used for tumor detection, and it is more efficient and cost-effective than X-ray detection. But this is a challenge for operators and requires well-trained operators to perform [1]. Meanwhile, the accuracy of ultrasound imaging is 17% higher than that of X-ray detection [2]. There is also a more high-precision technology called MRI, which performs better than the previous two technologies but is also very expensive.

However, when using these imaging techniques, collecting a large amount of image data is often necessary to obtain diagnostic image data through manual analysis. This significantly increases the workload of diagnostic personnel and greatly tests their professionalism. It has also increased many labor and time costs. In order to solve this situation, the application of AI in processing medical images is very important. Artificial intelligence is an important component of the intelligence discipline and an important tool for developing medical image processing towards intelligence. Research in this field includes robotics, language recognition, image recognition, natural language processing, and expert systems. Since its inception, artificial intelligence has become increasingly mature in theory and technology, and its application fields have expanded. Processing massive medical image data through AI has become a mainstream method and main tool, and has achieved very good results in disease diagnosis and prognosis detection [2]. On the one hand, the introduction of AI greatly reduces the workload of doctors. Moreover, when faced with subtle or blurry image information that is difficult for the human eye to recognize, AI's auxiliary diagnosis and image enhancement functions can also help doctors extract effective information from images, improving the accuracy of diagnosis [1]. On the other hand, a large amount of redundant, fuzzy, and irrelevant information is filtered by AI, which reduces the labor cost of medical treatment and generates excellent socio-economic benefits. This also improves doctors' diagnostic speed and alleviates the medical pressure on society.

This article will introduce and briefly summarize the new research and achievements of AI in medical image processing field since 2019, mainly in AI assisted diagnosis of breast cancer, and the diagnostic performance of the combination of AI and traditional medical imaging methods. This article will not delve too much into the specific technologies of AI in the research, but will focus on the effectiveness of AI application in this field.

2. Several diagnostic methods of breast cancer using AI

The AI technologies mentioned here mainly include machine learning (ML) and deep learning (DL). Machine learning is the process of automatically analyzing data through computers under supervised or unsupervised conditions, discovering certain patterns, and then using these patterns to predict the trend of data changes to guide production [4]. Generally speaking, the larger and more regular the dataset, the better the effectiveness of machine learning. Deep learning is a very important direction in machine learning. Deep learning aims to learn the deep rules of sample datasets, which can better process information in medical image signals. Ultimately, it achieves the function of discovering anomalies and diagnosing diseases like a doctor [4]. In the following, we will discuss in detail how artificial intelligence plays a role in the three main breast cancer diagnosis methods and gradually becomes an important part of modern medical imaging technology.

2.1. AI and mammography

The X-ray examination of the breast mainly uses a technique called breast molybdenum target for imaging examination. Breast molybdenum target imaging is basically not limited by age, and has high

repeatability, relatively high accuracy, and low cost, making it a non-invasive technology. In a study comparing the accuracy of molybdenum target and MRI, 50 patients with breast cancer and 60 patients with benign breast tumor were tested, and the accuracy reached 79.37% and 87.3% respectively [5].

The combination of AI technology and breast X-ray can improve the diagnostic speed and accuracy to a new level. A study on 114421 female breast X-ray image samples showed that the specificity of mammography with AI participation was only slightly improved compared to without AI participation. However, out of these 114421 images, 71585 were independently completed by AI without the participation of doctors, which means the workload of medical students was reduced by 63%. And 25% of false positives were avoided, with a total of 2107 false positives in all image samples, of which AI and misdiagnosis recognized 529 was avoided [6].

The main purpose of this study is to score each sample through AI. A low score is considered to be a low risk of breast cancer, rather than being sent to a doctor for examination. A high score is considered as a high risk of breast cancer and will be sent to the doctor for further examination. This kind of AI participation is to help doctors exclude people with low breast cancer risk, so that doctors only need to diagnose people with moderate or higher risk. Because the diagnostic difficulty of low-risk populations is low, AI can be used to avoid excessive occupation of doctors' time. The result of this method for the sample is that 791 of 114421 participants (excluding the abnormal part of the data, the abnormal part of the data has been eliminated) were diagnosed with breast cancer, 327 with interphase cancer, 1473 with long-term cancer, and 111830 with health. The probability of false positive in AI testing is about 2.5%, which is higher than the 1.8% false positive probability in manual testing. Figures 1 and 2 show the experimental results of the artificial group and the AI group, respectively [6].

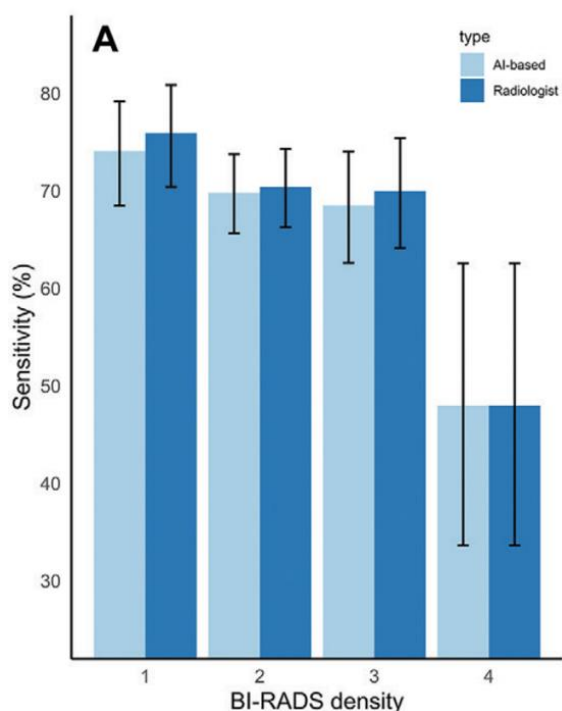


Figure 1. Bar graph shows a comparison of measured radiologist and artificial intelligence-based screening sensitivities [6].

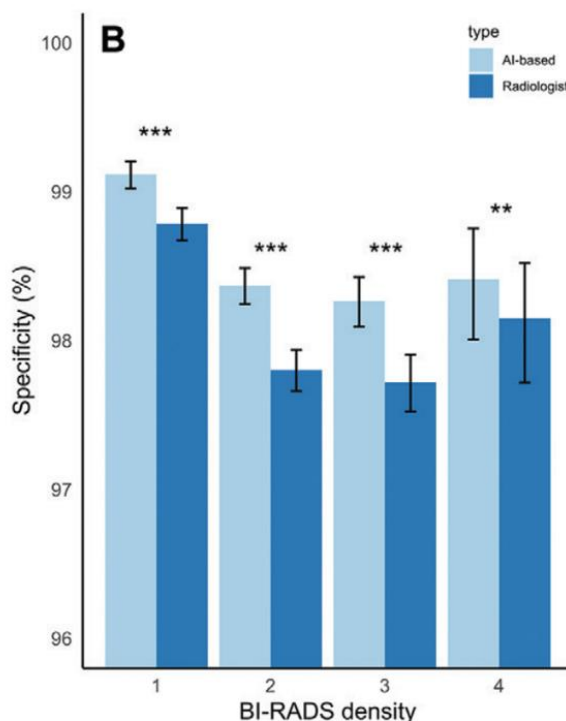


Figure 2. Bar graph shows a comparison of measured radiologist and AI-based screening specificities [6].

However, even though AI has made accurate diagnoses and predictions of the results, most AI cannot provide a corresponding and reasonable explanation for the diagnosis, which means that AI does not know the reason why they make such judgments themselves. Given that the medical field is

highly sensitive, all doctors cannot fully adopt AI diagnosis. So, allowing AI to use natural language to explain the reasons for diagnosis will undoubtedly accelerate the pace of intelligence in the medical field. The following will present a study on using AI to extract features of breast tumors and convert them into natural language output, which is to use human language to explain what information AI reads from images and make diagnoses [7].

This study used convolutional neural networks and deep neural networks to construct an AI model, but this article will focus on the data and results without excessively explaining the model. In this study, it is believed that breast cancer has a total of 21 characteristics, and the sample data provided includes 878 data, including 611 cases of sarcoma, that is, benign, and 267 cases of cancer, that is, malignant [7].

This study constructed a total of six different models and identified and explained these 21 features, ultimately resulting in a ranking table of the recognition efficiency of the six models for different features.

Table 1. Feature importance ranking [7].

Feature	RF	XGBoost	GBDT	SHAP	TabNet	Causal-TabNet
Skin	15	10	19	21	3	16
Areola	6	3	4	2	8	8
Nipple	16	11	14	11	10	19
Subcutaneous fat	20	12	21	12	16	20
Gland morphology	5	12	8	13	18	18
Gland edge	12	1	3	1	7	3
Increased gland density	10	14	20	10	4	14
Gland shape	19	15	16	15	14	9
Gland distribution	9	9	7	8	21	5
Calcification	21	16	18	16	20	13
Lesion type	13	5	15	6	2	8
Quantity	11	4	9	3	13	10
Position	1	7	1	7	11	17
Shape	2	8	2	5	9	21
Long side	7	6	11	9	5	4
Area	8	17	6	17	1	6
Aspect ratio	4	18	10	18	15	15
Boundary	18	19	13	19	19	1
Edge	3	20	5	20	12	11
Density	17	2	12	4	17	12
Lymph nodes	14	21	17	14	6	2

As shown in Table 1, a lot of information can be obtained. Different models have their advantage intervals, and combining multiple models can be used to achieve better recognition results. For example, boundary feature recognition of tumors is very important for the judgment of benign and malignant tumors, as the edges of benign tumors are smooth, while those of malignant tumors are sharp and have burrs. Finally, by outputting it into natural language, the interpretability of AI in medical image diagnosis can be improved. This interpretability increases the credibility of AI, makes

AI training easier, and reduces the workload of doctors, which is conducive to improving the social acceptance of artificial intelligence medicine [7].

2.2. AI and ultrasound

Compared with mammography, ultrasound examination for non-prophase breast cancer is more effective. Moreover, for some women with dense breast distribution, the misdiagnosis rate of breast X-ray is over 50%, while the accuracy and specificity of breast ultrasound are very good, and the results have high repeatability and relatively low cost [8]. As early as 2017, Han et al. trained a model that used convolutional neural networks to detect suspicious lesion areas and achieved good results [9]. This has proven the feasibility of AI and deep learning in assisting in diagnosing ultrasound images. Meanwhile, a contrast-enhanced ultrasound technique using micro bubble contrast agents can provide more detailed information than traditional B-mode ultrasound, including the morphological distribution of tumor blood vessels [10].

The following will describe a study on AI assisted diagnosis based on this microbubble contrast-enhanced ultrasound. These studies included 68 cancer cases and 119 benign tumor cases. By training AI models to analyze the image features of tumors and classify benign and malignant tumors. At the same time, there will also be professional doctors to classify AI, as a criterion for evaluating the accuracy of AI classification. This study used five AI models and three fusion models, and the diagnostic specificity working curve obtained is shown in the following figure [8].

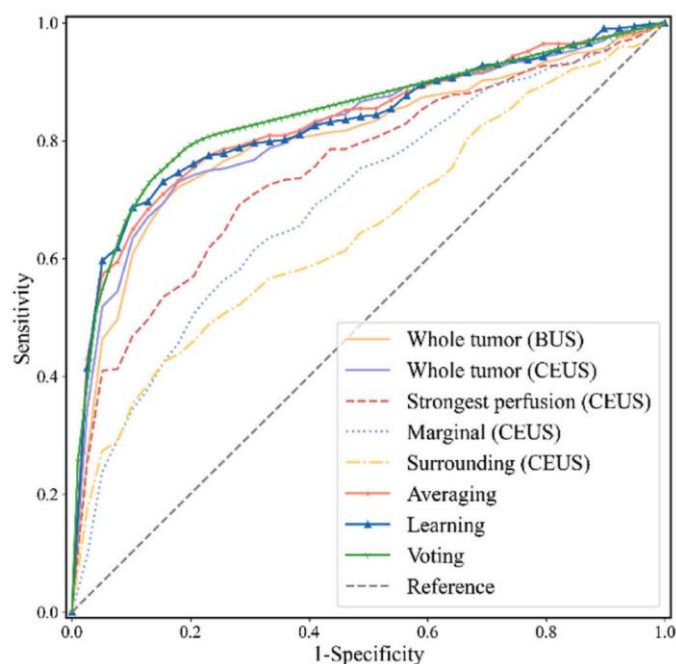


Figure 3. Receiver operating characteristic curves of five single-region models and three fusion models [8].

The data in Figure 3 shows the recognition and diagnostic efficiency of different models for different regions, as well as the diagnostic efficiency of the three fusion models. It can be concluded that the fusion model is usually better than the single model, in which the AI classification of multiple models for bisexual and malignant tumors has good development prospects. Here, the researcher also mentioned that such models may apply to the classification of breast cancer and other diseases that need to recognize image features. This AI assisted classification method has great help in improving medical conditions, especially in underdeveloped areas. However, this study also has a problem with a small sample size, and further validation of the model's feasibility is still needed.

However, there is a problem that deep learning requires a large number of breast cancer picture materials as a database to achieve good results. If doctors are asked to provide AI with artificial data sets, this will cause serious workload [11]. However, one study shows that metastatic learning can achieve good results in diagnosing breast cancer.

Transfer learning is also a method of deep learning, but it does not rely on a large amount of data like deep learning, and even if the dataset is nonlinear, transfer learning can achieve good results. Specifically, through previous learning, even if the previous learning is not closely related to the current task, transfer learning can inherit some experience and knowledge from the previous learning, thereby reducing the learning cost of the current task. Just like human learning, it is a continuous process. This technology is particularly helpful in digital signal and image processing [12].

The dataset for this transfer learning study comes from 100 images of benign tumors and 100 images of cancer in public databases. Two different transfer learning models are used for AI diagnosis: the traditional transfer learning model(CTL) and the multi-stage transfer learning model(MSTL). This study also used three convolutional neural network optimizers, SGD, Adam, and Adagrad. And compare the diagnostic performance of these two models, as shown in the figure [11].

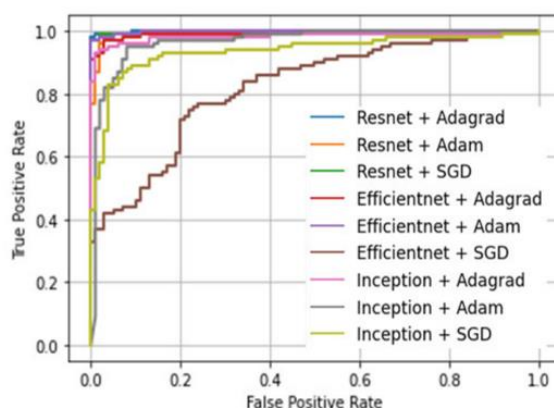


Figure 4. Multistage transfer learning ROC curve[11].

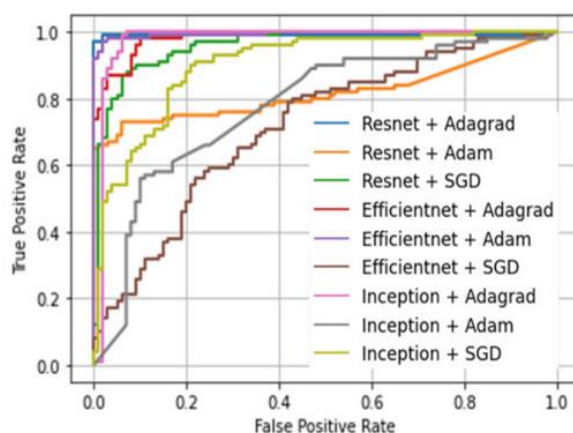


Figure 5. Conventional transfer learning ROC curve. SGD: Stochastic gradient descent[11].

The information read from the Figure 4 and Figure 5 found that the diagnostic performance of multi-stage transfer learning is significantly superior to traditional transfer learning. The significance of this study is that through a small number of breast cancer data sets, it has achieved superior diagnostic performance compared with other AI models in the same period. The cancer cell images used for transfer learning are easy to obtain and can be automatically preprocessed, so this project

reduces the time and labor costs required for model training and the requirements for hardware performance. And it can improve clinical decision-making, allowing patients to avoid suffering. However, there is currently a problem of AI suddenly forgetting the learning progress in transfer learning technology, which still needs improvement [11].

2.3. AI and MRI

MRI technology has a better accuracy rate in detecting breast tumors. In detecting 53 patients with breast cancer and 61 patients with benign breast tumors, the accuracy rate reached 84.13% and 90.48% respectively. If combined with other technologies, it will achieve a higher accuracy rate [5]. But MRI technology is often used for detecting severe cases due to its high cost. However, it can provide extremely high resolution, so MRI images are a suitable dataset for AI training [13].

The research introduced below also uses transfer learning methods to further improve AI-assisted MRI diagnosis's specificity. This study used five transfer learning AI models to analyze and diagnose three cancer features. The study sample was 174 breast cancer patients. Similar to the previous transfer learning, it also helps reduce the demand for sample size in the dataset through previous learning of AI. The final results of this study are shown in Figure 6 [14].

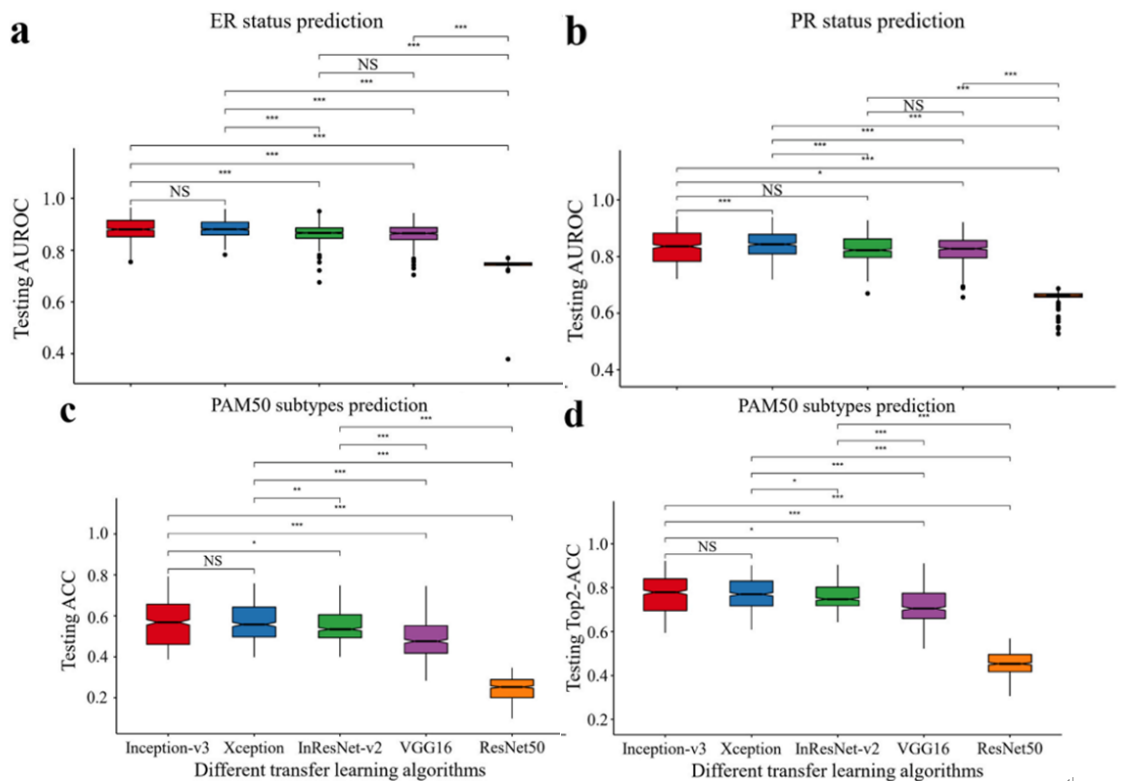


Figure 6. The impact of different transier learning algorithms on model performance [14]

Overall, we can see that the diagnostic performance of this exception model is better than other models. However, the diagnostic effect of PAM50 subtypes is not very good and cannot reach the medical level, even if it is superior to other models. Overall, this study can demonstrate the high medical value and feasibility of the xception model. Moreover, this study has low hardware performance requirements and even has the potential to be used on low-performing devices such as mobile phones [14]. At the same time, it also indicates that MRI technology can be combined with AI to assist medical diagnosis, which has good development prospects and may reduce the high cost of MRI technology.

This is significant for some women with a family history of breast cancer. The breast density of such people is higher than that of ordinary people, which means that the effect of ultrasound and X-ray is not good for such people. The advancement of MRI technology will benefit these people [13].

3. Conclusion

This article reviews and summarizes the research of AI in the field of auxiliary diagnosis of breast cancer in the past four years, and finds that AI has made some achievements in this field and has great potential for development. From the previous research data, some AI models reduced the workload of doctors by more than 60%, which greatly reduced the workload of doctors and improved the diagnostic efficiency of breast cancer. Moreover, breast cancer is a disease with a good early prognosis. Improving efficiency also reduces the cost of breast cancer prevention and increases social welfare. However, AI-assisted diagnosis of breast cancer is still not very mature, and many aspects must be improved. For example, a public breast cancer sample database can be built with the patient's permission to reduce the training cost of AI model and avoid AI training difficulties caused by insufficient data. Moreover, there is a slight gap in the accuracy and specificity of AI diagnosis compared to professional doctors, and it may be necessary to optimize the model's algorithm or a larger sample database to achieve higher accuracy and specificity. Building an open database also helps AI validate its diagnostic performance for later improvement. Finally, people are often very concerned about their own diseases and unwilling to let AI diagnose them. Therefore, after the technology matures, it is necessary to improve the social credibility of AI to reduce patients' resistance.

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Neural interface technology for human-computer interaction

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Abstract. Brain-Computer Interfaces (BCI) offer a direct communication channel between the brain and external devices, marking a pivotal convergence of neuroscience and technology. Neural activities, essentially electrical impulses, can be captured either invasively, with methods such as Electrocorticography (ECoG) which require surgical implantation, or non-invasively using techniques like Electroencephalography (EEG) that operate externally. Once acquired, raw neural data undergoes processing; external and physiological noises are filtered out, and meaningful patterns or neural ‘fingerprints’ are extracted. Modern BCIs then employ machine learning, specifically deep learning, to translate these cleaned neural patterns into discernible commands, with continuous feedback loops enhancing system adaptability. These decoded signals can control varied devices, from medical-grade robotic limbs to cursors on screens. BCIs have transformative applications across sectors: they’re pivotal in neurorehabilitation after brain injuries, providing feedback where traditional methods might fail; they’re integrated with virtual reality for immersive feedback; they revolutionize cognitive training and meditation practices; and they’re finding a foothold in high-risk sectors like deep-sea exploration and military operations. Additionally, research on tactile Event-Related Potential (ERP)-based BCIs emphasizes the importance of congruent Control-Display Mapping for efficient user experience. However, with these advancements come ethical concerns, such as the potential invasion of the privacy of one’s thoughts, challenges to human identity and autonomy, societal disparities in access, and health implications.

Keywords: Neural, BCIs, Human-Computer Interaction

1. Introduction

In a time characterized by relentless technological evolution, the pursuit of refining the interface between humans and their digital counterparts has consistently taken center stage [1]. Decades ago, traditional buttons and command lines were the norm in computer interactions. As technology matured, touchscreens and voice recognition paved the way, representing milestones drastically enhancing user interactions [2]. These mechanisms were not mere showcases of innovation; they addressed specific challenges, ensuring that interactions became more natural and intuitive, thereby setting the precedent for the next wave of advancements in this field. Today, as our society thirsts for even more seamless interactions with quicker real-time responses, the limelight shifts towards a once science-fiction domain: neural interfaces.

Neural interfaces, widely recognized as BCI, have a more profound history than most realize. BCIs trace their origins to the early experiments in the 1960s and 1970s when pioneering neuroscientists began to understand and decode the brain’s electrical rhythms [3]. By the 1970s, the first rudimentary

BCIs were helping simple computer cursors to move through brainwave modulation. These initial ventures paved the way for profound research and the realization that the human brain, with its billions of neurons and intricate networks, could potentially communicate with external devices without any physical intervention.

Over the subsequent decades, the research intensified, leading to sophisticated BCIs that leveraged advanced algorithms to interpret neural signals more accurately. As a result, the 21st century saw a surge in applications. Medical prosthetics became one of the earliest beneficiaries, as BCIs enabled individuals with paralysis or limb amputations to control artificial limbs or regain mobility. Outside the medical realm, the promise of BCIs extends into realms of everyday computing, enhancing virtual reality gaming experiences or as audacious as operating machinery solely through thought.

However, this transformative technology does not come without its share of challenges. As with many groundbreaking inventions in history, BCIs tread the fine line between the promise of a better future and the shadow of ethical dilemmas. These concerns range from privacy and security of one's thoughts to deeper philosophical debates about the nature of consciousness and the essence of humanity.

While the following sections will delve deeper into the intricate mechanisms behind BCIs, their diverse applications, and the ethical debates they evoke, it is crucial to appreciate the history and the journey that has brought us to the brink of such an exciting interface era.

2. Basic Mechanisms of BCIs

Niedermeyer and da Silva elucidated that all neural activities are electrical impulses that can be tapped into [4]. BCI have often been branded the frontier of neuroscience and technology's amalgamation. Essentially serving as conduits for direct communication between the brain and external devices, BCIs have the potential to revolutionize our interaction with technology. To understand BCIs' profound implications, dissecting their intricate mechanisms is crucial.

2.1. Signal Acquisition: The Beginning of the Journey

Every thought, feeling, and movement originates from a flurry of electrical impulses in the brain. Tapping into these impulses is the primary step for a BCI.

2.1.1. Invasive Methods. Directly interfacing with the brain, these methods are the gold standard for acquiring precise neural data. Techniques such as ECoG necessitate surgical implanting electrodes within the brain tissue or on its surface. The unparalleled signal quality justifies the invasiveness, making it invaluable in medical applications where precision is paramount.

2.1.2. Non-Invasive Methods. These methods, like the popular Electroencephalography (EEG) or Magnetoencephalography (MEG), are external to the body. Electrodes are placed on the scalp, capturing neural activity indirectly. Their non-intrusiveness makes them suitable for consumer-grade applications, albeit with a sacrifice in signal fidelity.

2.2. Signal Processing: Refining the Neural Code

The raw neural data, abundant with potential, is still a cacophony of signals. Extracting meaningful patterns necessitates refining this data.

2.2.1. Filtering. As external and physiological noises infiltrate raw neural data, it's imperative to isolate genuine brain activity, as highlighted by Blankertz et al. [5]. The brain is a bustling hub of activity. When first captured, Neural data is imbued with external interferences, from ambient electronic disturbances to physiological signals like muscle activity. Filtering algorithms meticulously isolate genuine brain activity from this noise, ensuring only pertinent data is utilized.

2.2.2. Feature Extraction. Specific patterns or signatures correspond to distinct intentions or thoughts among the cleaned neural data. Algorithms are deployed to identify these unique neural ‘fingerprints’. This process, known as feature extraction, isolates the most vital components of the neural data, streamlining it for translation.

2.3. Translation Algorithms: Decoding Intentions

With cleaned and streamlined data in tow, translating neural patterns into discernible commands begins.

2.3.1. Machine Learning & Pattern Recognition. Over the years, machine learning, especially deep learning, has emerged as the linchpin of BCI’s translation phase. Modern BCI systems leverage machine learning, as evidenced by LeCun et al., to correlate neural patterns with concrete commands [6]. These algorithms learn to correlate specific neural patterns with concrete commands by training on vast datasets. For instance, a specific sequence of neural activity might be decoded as the intention to move a cursor to the left on a screen.

2.3.2. Adaptability. A standout feature of modern BCIs is their adaptability. Continuous user feedback ensures these algorithms evolve, fine-tuning their accuracy. As the user interacts more with the BCI, the system becomes more adept at recognizing and translating their neural commands.

2.4. Output and Feedback: The Seamless BCI Loop

BCI’s heart lies the ability to transform abstract thoughts into tangible actions. Once the BCI interprets the user’s intentions, these decoded signals navigate many devices, from instigating movement in a medical-grade robotic limb to guiding a cursor on a screen in consumer applications. The success of this translation hinges on its immediacy and precision, as any delay or inconsistency can jeopardize user experience, rendering the interface ineffective. However, the BCI journey doesn’t end at actuation. The feedback loop is an essential component, seamlessly woven into the BCI ecosystem. As users witness the fruits of their neural commands, BCIs ensure they are apprised of the outcomes through varied mediums, visual cues, auditory signals, or tactile sensations. Beyond its informational role, this feedback mechanism fine-tunes user interaction, continually refining the alignment between user intentions and BCI responses, ensuring a harmonious and fluid experience. BCIs represent a dynamic dance of translation and feedback, orchestrating a symbiotic relationship between the human mind and technology.

3. Applications of Brain-Computer Interfaces

BCI, often envisioned as the vanguard of biotechnological advancements, are forging unprecedented pathways in human-machine collaboration. Beyond the conceptual intrigue, BCIs have spawned tangible applications that resonate across various facets of human experience. Delving into these applications offers a panorama of BCIs’ transformative potential.

3.1. BCI and Neurorehabilitation

As Dokkum et al. reported in *Brain computer interfaces for neurorehabilitation – its current status as a rehabilitation strategy post-stroke*, neurorehabilitation, particularly sensorimotor rehabilitation, is pivotal post-brain injury, aiming at restoring lost motor control to boost independence and quality of life [7]. Since stroke is the predominant cause of acquired disability in adults, it remains a focal point. With a substantial fraction of stroke survivors experiencing a deficit in motor functions, especially of the upper limb, it’s imperative to devise effective rehabilitative strategies.

Traditionally, many rehabilitation approaches have been rooted in motor learning theories, emphasizing the significance of “practice” in motor adaptation and decision-making. Key elements ensuring effective practice include repetitions, intensity, sensory priming, variability, and feedback. Feedback plays a dual role. It highlights performance areas for the patient and elevates engagement

and motivation. However, a challenge arises for those with severe deficits, as conventional tools require minimum motor control to extract feedback from therapeutic tasks.

Enter BCIs: These revolutionary interfaces decode and record brain activity during attempts at motor or cognitive tasks. BCIs have the potential to provide feedback, even when traditional methods fail, by mapping decoded brain signals onto sensory feedback, which can be visual, auditory, or haptic. Furthermore, this decoded signal can manipulate external devices to reproduce the intended movement, offering proprioceptive feedback. Essentially, BCIs serve as a bridge, extending therapeutic options to individuals regardless of their paresis severity.

A specific application of BCIs is combined with motor imagery (MI). MI is the mental rehearsal of a movement without physical execution. Before BCIs, monitoring kinaesthetic MI in patients was challenging. Now, by interfacing MI with BCIs, feedback can be visualized, enhancing motor learning and therapy engagement. For instance, Prasad et al.'s study combined physical practice with MI using a BCI interface, showcasing its feasibility in post-stroke protocols. Moreover, studies like those by Mihara et al. have illuminated the necessity of relevant feedback to induce desired neural plasticity.

The fusion of BCIs with virtual reality (VR) environments further amplifies their potential. Contrary to basic visual feedback, VR offers immersive 3D feedback, which can increase a patient's engagement. VR's capability extends to observational learning, with movement observation activating similar neural pathways as actual movement. Therefore, combining VR with BCIs allows patients to imagine and observe movements synergistically, further promoting motor learning.

Finally, BCIs offer the auxiliary advantage of brain activation monitoring during rehabilitation. Beyond their role in movement induction or MI feedback, BCIs can gauge global attention levels and monitor inter-hemispheric balance. For instance, assessing mental workload during BCI training can help optimize patient attention. Additionally, post-stroke inter-hemispheric imbalance information can guide treatment strategies.

BCIs herald a new era in neurorehabilitation, offering promising avenues for enhanced recovery, especially for those with significant motor deficits. Their integration with VR and capability for brain monitoring further cements their role as a cornerstone in post-brain injury care.

3.2. Cognitive Enhancement and Neurofeedback

The domain of cognitive training is witnessing an overhaul courtesy of BCIs. BCIs are transforming cognitive training, providing dynamic learning environments and aiding in meditation practices [8].

3.2.1. Dynamic Learning Environments. BCIs, when monitoring cognitive workload or focus, can modulate training material adaptively. For instance, if a learning platform detects dwindling attention, it might intersperse engaging multimedia or quizzes, optimizing information retention.

3.2.2. Meditation and Mindfulness. Neurofeedback devices, a subset of BCIs, are becoming popular in meditation circles. By providing real-time insights into brainwave states, these devices assist individuals in refining meditation techniques and navigating them towards desired mental states.

3.3. Industrial and Military Applications

High-risk sectors are leveraging BCIs for enhanced operational efficacy. BCIs are making strides in high-risk sectors, offering safer avenues for machine operation and military applications.

3.3.1. Machine Control in Hazardous Environments. In domains like deep-sea exploration or nuclear remediation, BCIs offer safer avenues for machinery operation. Operators, from a shielded locale, can mentally command drones or robots in hazardous zones.

3.3.2. Military Maneuvers. While rife with ethical considerations, defence sectors are intrigued by BCIs for drone operation, surveillance, and even potential communication applications that bypass conventional channels.

3.4. *Brain Mapping*

As Thurlings et al. reported in *Control-display Mapping in Brain-computer Interfaces*, BCI stand as the intersection between neurology and computation, and in the domain of this research, tactile Event-Related Potential (ERP)-based BCIs have taken the spotlight [9, 10]. This particular kind of BCI uses tactile stimuli, such as vibrations, to instigate navigation actions. A central research element was Control-Display Mapping (CDM), which can be succinctly described as the alignment between tactile stimuli and their visual display counterparts. The research bifurcated this mapping into two main categories: “Congruent,” where tactile controls and visual displays share the same orientation, and “Incongruent,” where there’s a mismatch, like a vertical display with horizontal controls. In their observations, congruent CDMs outshone their incongruent counterparts, boasting a significant 25% surge in performance efficiency. Furthermore, distinct neurological markers, namely the P300 and N2 components of ERP, were notably influenced by the congruency of the CDM. The heightened P300 in congruent setups implies a more focused attentional process, whereas an amplified N2 in incongruent scenarios suggests cognitive conflicts due to the misalignment of tactile and visual inputs.

The study’s findings aren’t merely academic; they possess tangible implications for the future design of BCIs. The pronounced P300 and its relationship with CDM hint at deeper cognitive processes tied to memory and recalibration, which are affected by the tactile-visual alignment. This realization pushes for a design ethos that emphasizes spatial congruency in CDMs, as this alignment not only streamlines the user experience but also enhances the functional capabilities of the BCI. By adhering to this recommendation, the end-user experiences fewer task errors, reduced mental strain, and optimized attentional resource allocation. In essence, for BCIs to achieve their peak potential in human-computer interactions, they should be designed with a harmonious alignment of tactile inputs and visual outputs, ensuring an intuitive and efficient experience.

4. **Ethical Implications of BCIs**

BCI, the brainchild of interdisciplinary research, are poised at the frontier of a technological renaissance. Yet, ethical questions arise as we approach this brave new era. Here, we delve into the intricate ethical tapestry associated with BCIs.

4.1. *Privacy, Security, and Integrity of the Self*

Sanctity of Innermost Thoughts and Vulnerabilities: BCI pose a dual challenge. Firstly, they may infringe on the final frontier of human privacy-our very thoughts. Deciphering neural patterns opens the door for potentially unauthorized access to emotions, memories, and desires. Secondly, like any digital apparatus, BCIs can be vulnerable to cyberattacks. However, the stakes are exponentially higher. A compromised BCI isn’t just about data; it’s about the sanctity of our mind. The thought of someone gaining unauthorized surveillance, or even the power to manipulate BCI-driven actions, is deeply unsettling.

4.2. *Identity, Autonomy, and the Ethos of Augmentation*

Human Identity Amidst Augmentation: BCIs, especially when applied to cognitive enhancement, force us to grapple with profound questions about our very identity. Coupled with this, is the challenge of autonomy. There’s a lurking danger as BCIs weave themselves into therapeutic or everyday settings. Over-reliance could lead to a diminished sense of agency, where individuals may begin to feel more “machine” than human. Moreover, informed consent in such a landscape becomes tricky. Understanding and consenting to BCI use requires a deep comprehension of potential, often unpredictable, long-term ramifications.

4.3. *Equity, Access, and the Ripple Effects on Society*

Societal Disparities and Cultural Divides: BCIs could inadvertently create two worlds: one of the augmented elite and the other of the augmented majority. Economic barriers might limit access to cutting-edge BCI tech, leading to “neural divides.” But disparities won’t stop at economics. Cultural

perceptions and regional regulations will play a significant role. While some societies might herald BCIs as the zenith of progress, others could deem them taboo, leading to fragmented global adoption. This divide, be it based on wealth, geography, or culture, could exacerbate existing societal inequalities, enhancing a privilege rather than a universally accessible tool.

4.4. Health, Societal Shifts, and the Evolution of Responsibility

Physiological and Societal Impacts: Delving into the unknown, especially with invasive BCIs, brings along a suite of concerns. Beyond the physical, there's the psychological dimension. The persistent interface of BCIs with the human mind could induce or exacerbate mental health challenges. But it's not just about individual health. BCIs could catalyze a seismic shift in how society functions. Imagine a world where verbal exchanges become secondary to direct neural transmissions. Such fundamental changes pose challenges of accountability. In scenarios where a BCI misinterprets a thought resulting in unintended actions, the lines of moral and legal responsibility blur.

5. Conclusion

BCI represent a profound biotechnological union, symbolizing the pinnacle of the fusion between man and machine. From their inception, capturing raw, intricate neural data to the tangible realization of a user's intention, BCIs are not just tools but powerful gateways. These gateways seamlessly link the intricate cerebral depths to manifest actions as bridges that turn aspirations into real-world outcomes.

As we peer into the future, several transformative developments beckon. The realm of medical science stands to gain immensely, with BCIs potentially enabling paralyzed individuals to regain mobility or those with speech impairments to communicate fluently. The education sector might witness revolutions, with BCIs facilitating rapid learning or even direct knowledge downloads. Our interaction with the digital world, be it in gaming or professional environments, will be redefined, offering unparalleled immersive experiences.

Integrating BCIs with other emerging technologies like AI opens doors to possibilities previously relegated to science fiction. Imagine a future where humans could collaborate with artificial intelligences in real-time through thought alone or where direct brain-to-brain communication reshapes the very essence of interpersonal relationships.

Yet, as we stand on the brink of an unprecedented era in human-machine interaction, it is evident that BCIs will become deeply ingrained in society's fabric, marking a transformative shift in our relationship with technology. However, the path forward isn't without its challenges. Beyond their impressive capabilities, BCIs introduce a host of ethical dilemmas, touching upon foundational issues of human morality, personal identity, and societal expectations. These range from the security and privacy of one's thoughts to the broader implications of enhanced cognitive abilities.

It's about mastering the technological aspects of BCIs and understanding and addressing the profound moral implications they introduce. As these interfaces become increasingly ubiquitous, fostering a comprehensive, ongoing ethical discourse becomes indispensable. It is a journey we must embark on with both enthusiasm and prudence, ensuring that as we harness the vast potential of BCIs, we remain anchored in our core values, preserving the essence of our humanity in a rapidly evolving digital age.

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The effectiveness and persistence of LTP on synaptic plasticity during sleep and sleep deprivation

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Abstract. The synaptic strength, which is crucial for memory and cognition function, is significantly influenced by sleep. According to earlier research, sound sleep is crucial for promoting connections between neural networks that are necessary for memory consolidation in the hippocampus. On the other hand, sleep deprivation can seriously impair the plasticity of our synapses. Sleep deprivation will disrupt LTP in region CA1 of the hippocampus, causing damage to neural connections. LTP has been proven to improve the connection between synapses, which will promote synaptic plasticity and so influence cognition function favorably. We also take a deeper look at LTP from a cellular and genetic perspective. In this review, we explore the detrimental effects of sleep deprivation on LTP stimulation and how they weaken synaptic plasticity and impair memory and learning performance. By studying LTP we can unravel the mysteries of brain function and the ability to learn and memory, and it can also give implications for understanding neurological disorders and advancing our knowledge of neuro system.

Keywords: Sleep, LTP, NMDA, AMPA, protein synthesis.

1. Introduction

In the past decade, there has been a growing body of evidence linking sleep to cognitive ability [1]. Sleep is an indispensable aspect of life, and sleep deprivation has been shown to diminish the biological capacity for information processing and memory consolidation. Numerous experiments have corroborated the significance of this effect in both animals and humans. In mammals, including humans, sleep patterns can be categorized into REM (rapid eye movement) sleep and non-REM sleep. It is believed that these two distinct sleep patterns exert differential influences on information processing and synaptic plasticity in the brain.

The consolidation and enhancement of memory during sleep primarily arise from synaptic modifications, with synaptic plasticity being closely associated with long-term potentiation (LTP) and long-term depression (LTD). This association stems from the synthesis of new proteins and synapses related to LTP. When individuals or animals experience sleep deprivation, LTP stimulation in the hippocampus becomes impaired, subsequently leading to reduced protein production as well as diminished synaptic plasticity—ultimately impacting memory formation and cognitive abilities.

However, it remains unresolved that which specific sleep pattern predominantly governs LTP induction and synaptic plasticity? whether it involves the forming of new NMDA receptors or AMPA receptors? Furthermore, how does LTP influence new protein synthesis? Is this process intricately linked

to protein synthesis? These questions persist unanswered. In order to solve these questions, multiple practical experimental methods will be used, including animal model and different tests. By using these methods the empirical and unbiased results will be acquired to better understand the function of LTP.

2. Part I experiment introduction

The first portion of the experiment will examine whether hippocampus long-term potentiation (LTP) will be hampered by REM or NREM sleep deprivation. In order to better understand how sleep deprivation, namely NREM and REM sleep loss, affects the brain's capacity to create and maintain connections in the hippocampus—a vital area for learning and memory—this study will assist. The goal of this article is to acquire a greater knowledge of how various sleep phases affect cognitive functions by looking at how these two forms of sleep deprivation affect LTP. NREM and REM sleep both have been demonstrated to be crucial for memory consolidation in earlier research, but it is still unclear how each of these sleep cycles contributes specifically to the process.

2.1. Methodology of Part I experiment

2.1.1. Animal Model. To conduct this study, Animal models was chosen such as mouse. Mice will be individually housed in cages under 12h light-dark cycle at room temperature (26°C). Then mice will be divided into three groups, labelled control group, REMSD (REM sleep deprivation) group and NREMSD (NREM sleep deprivation) group and will be implanted with devices to test hippocampal EEG signal. After the experiment begin, the mice in control group will remain normal sleep and wake up time, mice in REMSD group will be immediately waken up when they enter REM sleep stage, and mice in NREMSD group will be immediately waken up when they enter NREM sleep stage, and this will be repeated during night cycle (mice will be first exposed to light to live normally for 10 hours then start experiment).

2.1.2. Multiple Platform Method (REM sleep deprivation). To accomplish sleep deprivation on mice, the modified multiple platform approach was used [2]. Mice were kept separately in cages with 12 tiny, cylindrical platforms that sized 3.5 cm in diameter and 3 cm in height, submerged in water that was 1 cm deep. Animals were kept on platforms with free access to water and food pellets. They were startled by falling into the water as REM sleep progressed as a result of muscular atonia [3].

2.1.3. Gentle Handling Model (NREM sleep deprivation). Partial SD models provide a practical way to abruptly abolish portions of the sleep cycle for brief periods of time, despite the fact that it is almost difficult to deliberately block one particular phase or stage of sleep without impacting the other. When animals enter a certain sleep phase (REM or NREM), which is being tracked by EEG recordings or ocular observation, the gentle handling paradigm comprises providing tactile, olfactory, or visual stimulation [4]. Because it needs constant monitoring by the researchers, this paradigm is only helpful for brief periods of time.

2.1.4. Passive Avoidance Test. The passive avoidance test, which makes use of a shuttle box avoidance system, was used to examine mice's capacity to learn and retain stimuli like foot shock. The shuttle box had sections that were both light and dark, as well as a grid floor that was connected to an electric shock source. Mice were placed in the bright compartment after the experiment started, and the door between the two compartments was left open for 10 minutes while the mice explored the box. As soon as the animal entered the dark chamber, the door between the two compartments was shut, and a single electric foot shock (0.7 mA, 1 s) was administered for the purpose of measuring learning. In order to help the mouse connect the dark compartment with fear (foot shock), the mouse was remained there for an additional minute before being taken back to its cage at home. The mouse was placed back in the light room an hour after the initial foot shock, and the step-through latency (the amount of time it took the mouse to enter the dark chamber) was timed. Each set of mice had a retention time to step-through that

was recorded. After the mice entered the dark compartment, a second electric shock was administered. Mice were once more placed in the lit compartment after another hour in order to assess the step-through latency maximum of 300 s [5]. Compared to longer latency intervals, shorter latency durations signify poor memory recall.

2.1.5. MWM Test. In order to help the mouse connect the dark compartment with fear (foot shock), the mouse was remained there for an additional minute before being taken back to its cage at home. The mouse was placed back in the light room an hour after the initial foot shock, and the step-through latency (the amount of time it took the mouse to enter the dark chamber) was timed. Each set of mice had a retention time to step-through that was recorded. After the mice entered the dark compartment, a second electric shock was administered. Mice were once more placed in the lit compartment after another hour in order to assess the step-through latency maximum of 300 s. Compared to longer latency intervals, shorter latency durations signify poor memory recall. Visible platform training was used for the pre-training water maze exam. At the intersection of the four quadrants, 0.5 cm above the water's surface, a white, cylindrical platform with a diameter of 6 cm was positioned. After being released at random into each of the four quadrants, mice were given two minutes to swim to the platform. Days 1 through 5 (P1–P5) of concealed platform training involved moving the platform to a random place and submerging it 1 cm beneath the water's surface. Each mouse was launched randomly and in a different direction. Every day, training sessions were run with a 30-min break in between, and the latency to each platform was recorded. The platform was taken out of the concealed platform test the day after the last day (day 6), and mice were given 60 seconds to investigate the pool as part of a probing trial. The platform's former location's opposite quadrant saw the discharge of mice. Time spent swimming in each of the four quadrants was noted. To assess memory retention, it was calculated the proportion of time spent in the target quadrant [6].

2.2. Results and discussion of Part I experiment

Using the multiple platform method and gentle handling model, the paper successfully induced REM sleep deprivation and NREM sleep deprivation on mice and then used passive avoidance test and MWM test to measure the effects of SD on mice's ability to learn and remember and their spatial memory. As the results shown, mice with REMSD entered the dark chamber with shorter retention time compared to control group and mice with NREMSD. In the first session of the MWM test, mice from all three groups arrived at the escape platform at around the same times. In sessions 2, 3, 4, and 5, the REMSD group took longer to reach the escape platform since the escape latency time significantly reduced with the number of sessions. As a result, we can say that REMSD primarily hinders animals' learning and memory functions. It can also reduce LTP. As was mentioned in one article, REM sleep plays a role in maintaining synaptic plastic changes in the PP-DG circuit, whereas REMSD after LTP induction severely hinders the maintenance of LTP in the PP-DG pathway [7].

3. Part II experiment introduction

In the second part, the paper will continue to investigate the relationship between sleep and LTP, and my exploration will extend to focusing on NMDA receptors and AMPA receptors and their impact on sleep. These two types of glutamate receptors are fundamental components of synaptic plasticity, which underlies learning and memory processes in the brain, and also are indicators of LTP stimulation. Due to limitation of our lab equipment, the paper will only test on the enhancement of NMDA receptors on sleep. By examining how NMDA receptors impacts sleep, insights into the mechanisms can be acquired through which sleep promotes synaptic strengthening.

3.1. Methodology of part II experiment

To conduct this study, the participants were divided into two groups, one as control group. Both groups were assigned to a learning and memory task at 10p.m. and they will be taught for 1.5 hours. Two groups of participants were advised to retire to bed at 11:30, and the experiment group was given D-cycloserine

(DCS), a coagonist at the NMDA receptor's glycine binding site that helped declarative memory encoding [8], just before the lights went off. Both groups were awakened up after 7.5 hours of sleep, and their memory functions were then assessed.

The declarative word-pair association task was used to evaluate the participants' memory and capacity for learning after sleeping [9]. First, the participants had to recall a list of 40 word pairings. Performance was evaluated utilizing a cued-recall approach following the display of the whole list, which included feedback of the right word. The cued-recall process was repeated until the individual met the 60% accurate response threshold. The same cued-recall approach used throughout the learning phase was used to measure retrieval at the conclusion of the experimental session. As a gauge of nighttime retention, the absolute disparities between word pairs that were remembered during retrieval testing and on the criterion trial during learning were used.

3.2. Results and discussion of part II experiment

By using this memory test, the paper demonstrated the influence of NMDA receptors on synaptic plasticity during sleep. Experiment result showed that DCS administration before sleep distinctly improved the recall of word pairs at the last session of experiment, and this was essential evidence for the importance of NMDA receptor in consolidation of memory. During REM sleep when brain engages in consolidating newly acquired information into solid memory, the activation of NMDA receptors within hippocampus facilitates this process by strengthening synaptic connections between neuron, more specifically speaking, by enhancing LTP.

4. Part III experiment introduction

In the third part, the relation of LTP and new protein synthesis will be discussed. LTP is a cellular model for many forms of learning and memory in hippocampus, and to ensure its stabilization and persistence, the synthesis of novel proteins becomes essential [10]. These newly synthesized proteins are involved in gene expression and contribute to the structural changes at synapses necessary for long-term memory formation. They will also play other important roles such as modulating neurotransmitter release and receptor trafficking during LTP expression, so we can say to some extent LTP is protein-synthesis dependent [11].

4.1. Methodology of part III experiment

Transverse hippocampus slices from rats were cut using a McIlwain tissue cutter to demonstrate this theory. Following 20 minutes of steady recording, LTP was generated by two 100 Hz, 1 s strand, 20 s apart, at a current strength to elicit field excitatory postsynaptic potentials (EPSP), which were recorded using glass microelectrodes soaked in saline solution and positioned in the stratum radiatum. Then, one set of the test slices was kept in rapamycin, an inhibitor of growth-related protein synthesis, throughout the duration of the recordings [12]. To investigate if LTP is dependent on novo protein synthesis, the intensity of EPSPs will be continually measured.

4.2. Results and discussion of part III experiment

During stable recording, EPSPs of both groups were almost the same, and the readings were around 100% baseline. When LTP was induced, EPSPs of both groups increased rapidly to 150%, but after incubating experiment slices into rapamycin, EPSPs of experiment group decreased gradually to 110% in 80 min, however EPSPs of control group stayed at 150%. This result gave us evidence that LTP in hippocampus requires new protein synthesis to remain its persistence.

5. Conclusion

In general, this review explored the connection between sleep, long-term potentiation (LTP), NMDA receptors and protein synthesis, providing an overall view of how these factors intertwine to impact cognitive function and memory. Sleep, which is an indispensable process of life, plays a pivotal role in consolidating memories and strengthening synaptic connections, primarily through LTP and LTD. On

the contrary, sleep deprivation has detrimental effects on LTP, and the results from first part demonstrated that REM sleep deprivation prominently impairs learning and memory processes, suggesting a critical linking between REM sleep and LTP, which also in accord with previous research that REM sleep is important in maintaining high synaptic plasticity. After that, NMDA receptors were proved to strengthen consolidation of memory and enhance LTP function, and LTP was closely dependent on new protein synthesis. From this review we know adequate sleep supports memory consolidation, while sleep deprivation hinders LTP function and thus impair synaptic connections. NMDA receptors and protein synthesis mechanisms provide us cellular and genetic view of this process and give us a deeper understanding of how our brain encode and retain information. This research advances our knowledge of fundamental neurobiology and also highlights the importance of healthy sleep patterns to our cognitive function and memory retention. However, this paper still maintains some deficiency, such as lack of repeated experimentation or limited experiment methods and research targets. For example, the paper only used mouse as model to perform the experiment and all the results were acquired from this single animal model. For future experiments, more models are expected and the results from different models can be analyzed and a more comprehensive conclusion can be made. The future research directions in the field of LTP has been evolved since then, and I think the potential areas of interest may focus on the mechanism of LTP induction, and the relation of LTP to disease. By examining the mechanism of initiation of LTP and the role of LTP in many neurological disorders such as Alzheimer's Disease and Depression, we can better know our brain and advance medical development.

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