TNS

Theoretical and Natural Science

The 3rd International Conference
on Biological Engineering and Medical Science

Oxford, UK

September 2 - September 9, 2023

EWA Publishing

Volume 21

Editor
Alan Wang
University of Auckland

ISSN: 2753-8818
ISSN: 2753-8826 (eBook)

ISBN: 978-1-83558-215-2
ISBN: 978-1-83558-216-9 (eBook)

Publication of record for individual papers is online:
https://tns.ewapublishing.org/

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Address: John Eccles House, Robert Robinson Avenue, Oxford, England, OX4 4GP
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Preface

The 3rd International Conference on Biological Engineering and Medical Science (ICBioMed 2023) is an annual conference focusing on research areas including biological engineering, biomedical engineering, bioinformatics, basic science of medicine, clinical 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 biological engineering, biomedical engineering, bioinformatics, basic science of medicine, clinical and public health.

This volume contains the papers of the 3rd International Conference on Biological Engineering and Medical Science (ICBioMed 2023). 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, ICBioMed 2023 organized five workshops in Auckland, Birmingham, Eskisehir, Kuala Lumpur and Petaling Jaya. Dr. Alan Wang chaired the workshop "Workshop on Neuroimaging Quantification for Precision Medicine 2023 (WNQPM 2023)", which was held at The University of Auckland. Dr. Maher G. Nawaf chaired the workshop "Immunotherapy with Chimeric Regulatory T Cells (CAR-Tregs)", which was held at University of Birmingham. Dr. Ömer Burak İSTANBULLU chaired the workshop "Computational Modelling and Experimental Analysis of Galvanic Corrosion Process and Characterization of the Implantable Biomaterials", which was held at Eskişehir Osmangazi University. Dr. Sathiya Prakash Sooryanarayana chaired the workshop "Refresher Course on Detection and Management of Diabetic Retinopathy among Diabetes Patients" at UCSI University. Dr. Sheiladevi Sukumaran chaired the workshop "Disability Etiology: Unpacking the Complexities for Effective Prevention and Treatment" at SEGi University.

Besides these workshops, ICBioMed 2023 also held an online session. Eminent professors from top universities worldwide were invited to deliver keynote speeches in this online session, including Dr. Andre Levchenko from Yale University, Dr. aher G. Nawaf from University of Birmingham and Dr Cristina Tudoran from University of Medicine and Pharmacy "Victor Babeș" Timișoara. They have given keynote speeches on related topics of biological engineering, biomedical engineering, bioinformatics, basic science of medicine, clinical 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 ICBioMed 2023, 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 ICBioMed 2023.

Andre Levchenko

Alan Wang

General Chairs of Conference Committee

Workshop

Workshop – Auckland: Workshop on Neuroimaging Quantification for Precision Medicine 2023 (WNQPM 2023)

August 18th, 2023 (GMT+12)

Faculty of Medical and Health Sciences and Bioengineering Institute, The University of Auckland

Workshop Chair: Dr. Alan Wang, Associate Professor in The University of Auckland

Workshop – Birmingham: Immunotherapy with Chimeric Regulatory T Cells (CAR-Tregs)

August 21st, 2023 (GMT+1)

Immunology and Immunotherapy Department, University of Birmingham

Workshop Chair: Dr. Maher G. Nawaf, Research Fellow in University of Birmingham

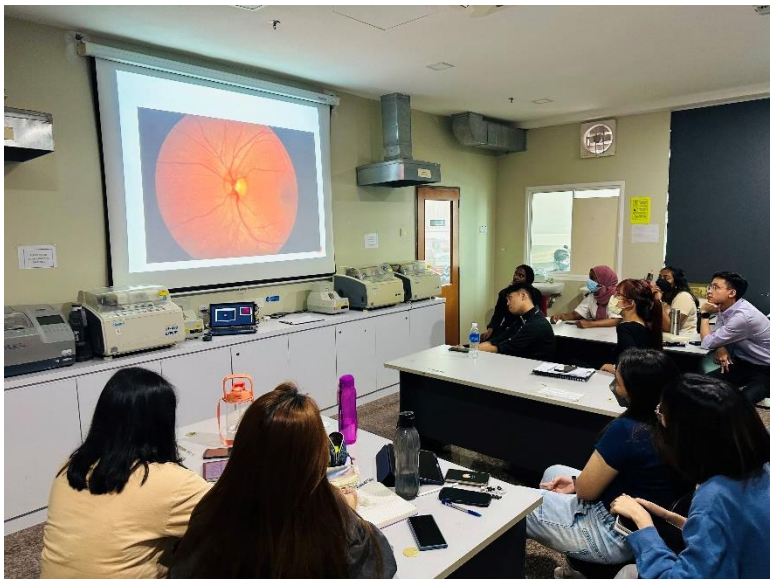
Workshop – Eskisehir: Computational Modelling and Experimental Analysis of Galvanic Corrosion Process and Characterization of the Implantable Biomaterials

June 30th, 2023 (GMT+3)

Department of Biomedical Engineering, Faculty of Engineering and Architecture, Eskişehir Osmangazi University

Workshop Chair: Dr. Ömer Burak İSTANBULLU, Assistant Professor in Eskişehir Osmangazi University

Workshop – Kuala Lumpur: Refresher Course on Detection and Management of Diabetic Retinopathy among Diabetes Patients



August 11th, 2023 (GMT+8)

School of Optometry, Faculty of Medicine & Health Sciences, UCSI University

Workshop Chair: Dr. Sathiya Prakash Sooryanarayana, Lecturer in UCSI University

Workshop – Petaling Jaya: Disability Etiology: Unpacking the Complexities for Effective Prevention and Treatment

September 1st, 2023 (GMT+8)

Faculty of Education, Languages, Psychology and Music, SEGi University

Workshop Chair: Dr. Sheiladevi Sukumaran, Associate Professor in SEGi University

The 3rd International Conference on Biological Engineering and Medical Science

ICBioMed 2023

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
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Forecasting the number of new crown infections in China based on machine learning methods

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Abstract. Based on the current state and the evolution of the new crown epidemic in China, this paper uses machine learning models and historical data to predict the changes in the number of new crown infections in China in the next four months. First, analyzing the background and current situation of the new crown epidemic, and identified the research question by collecting relevant historical data, including indicators such as the number of infected people, the number of cured people, and the number of deaths. Second, employing machine learning models and MIR model to predict the trend and scale of the number of new crown infections in China over the next four months. Finally, coming to a forecast conclusion: in the next four months, the number of new crown infections in China will drop very slightly (almost remain unchanged) every month, and the monthly infection rate will remain at a low level. At the same time, discussing and summarizing the application value of the conclusions. The research results of this paper can provide useful references and guidance for government policymakers and the public, helping them better deal with the epidemic and formulate corresponding measures. In addition, the research methods and models in this paper also have a certain degree of versatility, which can provide a certain reference for other countries and regions to predict the trend and scale of the new crown epidemic.

Keywords: prediction, infections, model.

1. Introduction

China was one of the first countries to be infected by 2019-nCoV, and since December 2019, more than 1 million cases of 2019-nCoV infection have been confirmed in mainland China [1]. In China, the spread of COVID-19 has caused many social problems such as shortage of medical resources, shortage of medicines, and logistics bottlenecks. These problems have seriously affected the development of my country's economy, education and culture [2]. Take education: Many schools and universities have closed their campuses in favor of distance learning or hybrid models. This brings additional burdens and challenges to those districts and students with limited educational resources [3].

Now this study predicting the number of new crown infections has many implications: First, it helps the government and public health institutions guide resource allocation, improve medical efficiency, maximize the use of existing resources, and protect public health. Second, predicting the number of future infections can make the public more aware of the severity of the epidemic and further strengthen

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DOI: 10.54254/2753-8818/21/20230799

self-protection and social responsibility. Furthermore, enterprises can adjust their production and operation strategies based on the forecast results, including taking measures to reduce the impact of the epidemic on the enterprise, adjusting product production and sales strategies, and improving the enterprise's ability to adapt and respond.

Having said that, to predict the number of new crown infections in the future, the paper need to use machine learning methods. Machine learning methods have produced impressive outcomes in many areas of forecasting. Such as image recognition and computer vision: Machine learning algorithms have shown amazing results in picture categorization, object detection, face recognition, etc., such as convolutional neural network (CNN) and recurrent neural network (RNN) in deep learning models; natural language Processing: Machine learning technology has made significant progress in speech recognition, text classification, machine translation, language generation, etc., such as recurrent neural network (RNN) and transformer model (Transformer); Finance: machine learning algorithms in stock forecasting, risk management Remarkable results have been achieved in, credit evaluation, market forecasting, etc., such as Random Forest (Random Forest), Support Vector Machines (Support Vector Machines) and Neural Networks (Neural Networks) [4-7]. Medicine and biology: Machine learning technology has achieved remarkable results in bioinformatics, medical image analysis, disease prediction, etc., such as convolutional neural networks (CNN) and recurrent neural networks (RNN) in deep learning models [8].

This is just a small sample of the applications of machine learning in different fields. Given the ongoing development of technology and the ongoing expansion of data, the scope of application of machine learning will become more and more extensive.

Furthermore, the steps of forecast research in this paper are as follows:

Data collection, cleaning and processing are carried out first. Then choose a machine learning model (when choosing a model, pay attention to choosing a model with high accuracy and generalization performance). Then build the model. Finally, the most important step is to perform model training, prediction and result visualization.

And in the following, the framework arrangement will be discussed separately: model, data, results, conclusion**.** It is predicted that in the next four months, the number of people infected with COVID-19 in China may decline slightly compared with the previous months, and the number of individuals who have COVID-19 will remain at a low level. So, government can continue to carry out epidemic monitoring and early warning; individuals can maintain personal hygiene habits, actively vaccinate, avoid gathering and cross infection and cultivate a good lifestyle.

2. Model

2.1. ML model

The main idea of machine learning (ML) is to allow computers to automatically complete tasks by learning patterns and regularities in data sets, without explicitly writing specific programs. The process involves using mathematical algorithms to extract features and patterns from data, and then training a model to predict outcomes on new data.

ML model has many advantages. Firstly, adaptability: ML models can adaptively change their behavior to adapt to new data inputs and changes in the environment. Secondly, high accuracy: ML models can improve the accuracy of predictions by learning from large-scale datasets, especially in complex tasks. Thirdly, automation: ML models can automate tasks, reducing the need for human intervention and increasing efficiency. It is usually applied to various tasks, such as image recognition, speech recognition, natural language processing, recommendation system, etc.

The calculation formula of an ML model depends on the algorithm and model type used. For example, in a linear regression model, the calculation formula is:

$$y = mx + b \tag{1}$$

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y represents the target variable (the variable to be predicted), x represents the independent variable (the variable used to predict the target variable), m represents the slope, and b represents the intercept distance. Therefore, the calculation formula of the ML model will vary with different algorithms and models.

2.2. SIR model

The SIR model is a mathematical model used to describe the spread of infectious diseases. It divides the population into three transitional states: Susceptible, Infectious, and Recovered. In the COVID-19 epidemic, the SIR model is widely used in epidemic prediction and epidemic prevention decision-making. The main idea of this model is to study the spread and control of infectious diseases in the crowd by modeling the flow and interaction of the crowd. It's simple, clear and easy to implement and understand.

SIR Model is commonly used to predict the transmission trend of infectious diseases, develop effective measures for preventing and controlling outbreaks, and evaluate interventions such as vaccination and isolation. Or it can be used to study other types of dynamic processes, such as social networks, epidemiology, and economics.

Calculation formula:

$$\frac{dS}{dt} = -\beta SI \tag{2}$$

$$\frac{dI}{dt} = \beta SI - \gamma I \tag{3}$$

$$\frac{dR}{dt} = \gamma I \tag{4}$$

S means the number of susceptible people, I means the number of infected people, R means the number of recovered people, β means how many susceptible people each infected person can infect every day, γ means each infected person can recover or die every day The probability. These parameters can be adjusted according to disease characteristics and transmission [9, 10].

2.3. Linear regression model

The main idea is to build a linear model to describe the relationship between independent and dependent variables, and use this model to predict new dependent variable values. It has many advantages: easy to understand and implement, high computational efficiency, good explainability, etc.

Calculation formula [11, 12]:

$$y = bx + a \tag{5}$$

$$b = \frac{n\sum_{i=1}^{n} XiYi - (\sum_{i=1}^{n} Xi)(\sum_{i=1}^{n} Yi)}{\sum_{i=1}^{n} Xi^2 - \left(\sum_{i=1}^{n} [\![Xi)]\!]\right.} \tag{6}$$

$$a = y - bx \tag{7}$$

2.4. Prediction model

2.4.1. MSE. Mean square error is a common index for evaluating the accuracy of regression model forecasts. It calculates the square of the average difference between the predicted value and the actual value. Specifically, the smaller the mean square error is, the closer the predicted results of the model are to the actual results. The calculation method of mean square error is as follows:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2 \tag{8}$$

Where n means sample size, yi represents the actual value.

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2.4.2. Accuracy. Accuracy is an indicator for assessing the accuracy of predictions in classification models. It calculates the ratio of the correctly forecast sample count to the total sample count. Specifically, the higher the accuracy, the closer the model prediction results are to the actual results. The accuracy is calculated as follows:

$$Accuracy = \frac{TP + TN}{TP + FN + FP + TN} \tag{9}$$

TP represents the number of true cases, TN represents the number of true negative cases, FP represents the number of false positive cases, and FN represents the number of false negative cases.

2.4.3. R-square. R-square is a widely used indicator for estimating the prediction accuracy of regression models. It calculates the proportion of the model prediction results that can explain the actual data variance. Specifically, the higher the R-square, the more integrated the data can be, and the closer the prediction result is to the actual result. The calculation method of R square is as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(Y_i - \widehat{Y}_i\right)^2}{\sum_{i=1}^{n} (Y_i - \overline{Y}_i)^2} \tag{10}$$

n represents the number of samples," Yi" represents the actual value of the ith sample.

2.4.4. RMSE. RMSE is the abbreviation of Root Mean Squared Error and the square root of MSE. The calculation formula of RMSE is:

$$RMSE = sqrt\,(MSE) \tag{11}$$

2.4.5. MAE. MAE is the abbreviation of Mean Absolute Error. It is an indicator used to measure the prediction error of the model, usually used for regression problems. MAE represents the average of the absolute value of the difference between the predicted value and the true value. The smaller the MAE, the smaller the difference between predictive model outcomes and actual outcomes.

The calculation formula of MAE:

$$MAE = \left(\frac{1}{n}\right) * \Sigma |yi - \hat{y}i| \tag{12}$$

n is the sample count, yi represents true value, ŷ represents the anticipated model value.

3. Data

This article uses the statistics of the number of people infected with the new crown epidemic from the Official website of the World Health Organization, and first selects the number of COVID-19-infected people in China from international data centers in countries, then Select the data after November 2022, and only analyze the data after November 2022.

3.1. Descriptive statistics of data

Table 1. Descriptive statistics.

Number of samples	Mean	Maximum	Minimum	Median	Cumulative case
133	796329	6966046	0	27606	98932687

Table 1 proves that there are enough samples in this study, and the values of Median and Cumulative case are also very real and reliable, which can be used for rigorous research and demonstration.

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3.2. Data visualization with SIR model

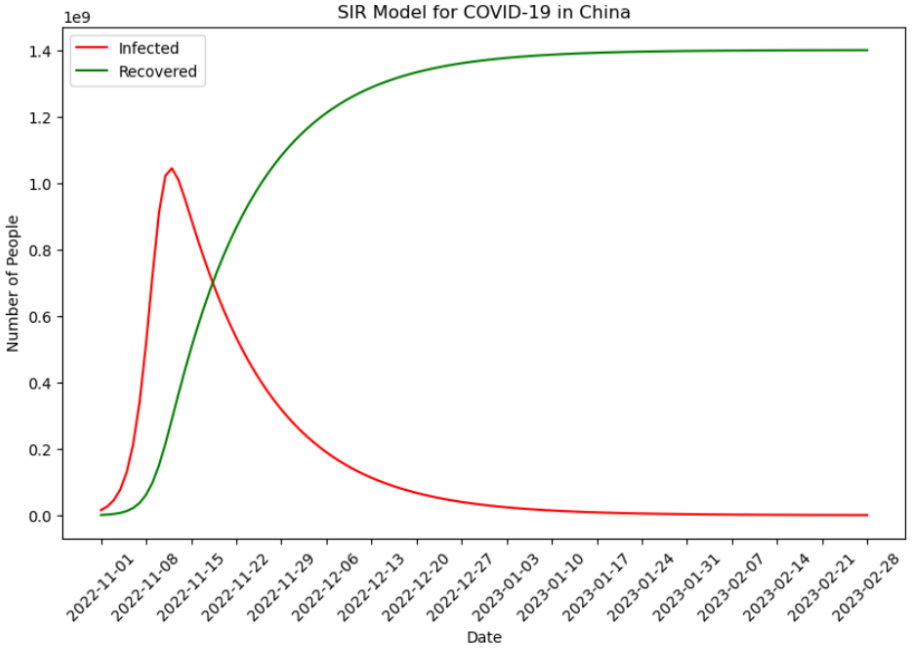

Figure 1. SIR Model for COVID-19 in China.

SIR is an infectious disease model, which is used to describe the mode of disease transmission in the population. The name of the SIR model comes from its classification of population into three categories: Susceptible, Infected and Recovered.

From Figure 1, it can be seen that the number of new cases of new crown infections in China rose sharply in early November, then dropped sharply in the second half of November, and then dropped back to a normal low value. Many of those infected with the new crown recovered quickly.

4. Result

4.1. Analysis of previous data

Previous data show that since the end of 2020, COVID-19 has spread rapidly around the world. Through strong measures and extensive publicity campaigns, China gradually liberalized epidemic management in early November 2022, making positive contributions to economic recovery and social normalization. According to the data, the number of COVID-19 infections increased rapidly when China just opened the epidemic control, but after the peak of the first round of infection, the number of new infections has been declining The data shows that China has achieved some success in controlling the epidemic, and the growth rate of the cumulative number of cases has slowed down. Although new cases continue to appear, the overall trend is to gradually improve.

4.2. Analysis of forecast data

The forecast data shows that in the coming months, China's COVID-19 epidemic may drop slightly or remain almost at the same value, which also shows that the Chinese government's decision to gradually liberalize epidemic management in early November 2022 is wise. The forecast data shows a relatively flat curve, indicating that the epidemic prevention measures taken by the Chinese government are effective and have mitigated the impact of the epidemic to a certain extent.

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4.3. *Data prediction with SIR model*

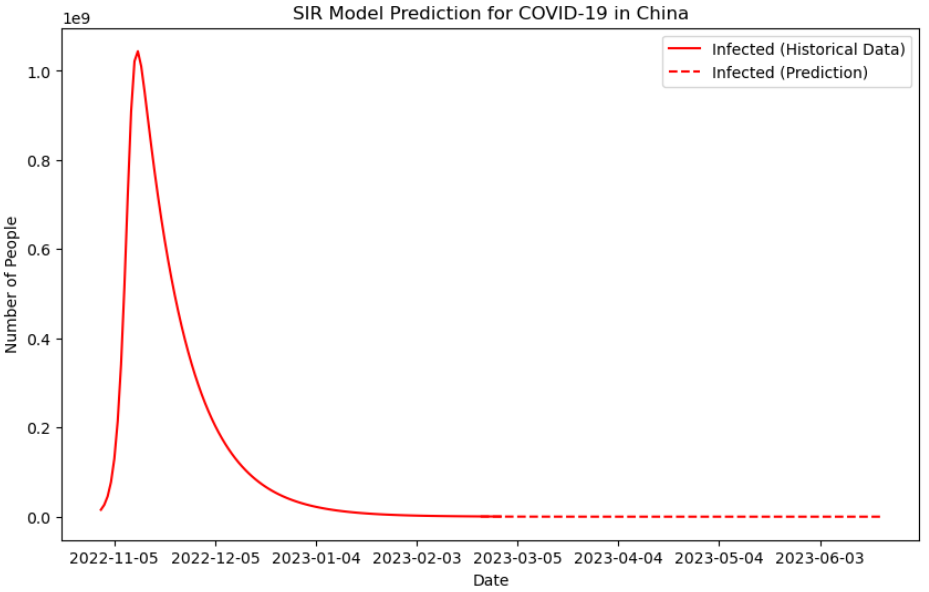

Figure 2. SIR Model Prediction for COVID-19 in China.

The solid line in the first half of Figure 2 is the same statistical data as in Figure 1 in the previous months, and the dotted line at the end is the forecast data for the number of new crown infections from March to June 2023. What is shown here is that the number of new crown infections will continue to be kept at a low value in the future.

4.4. *Data Prediction with Linear Regression Model*

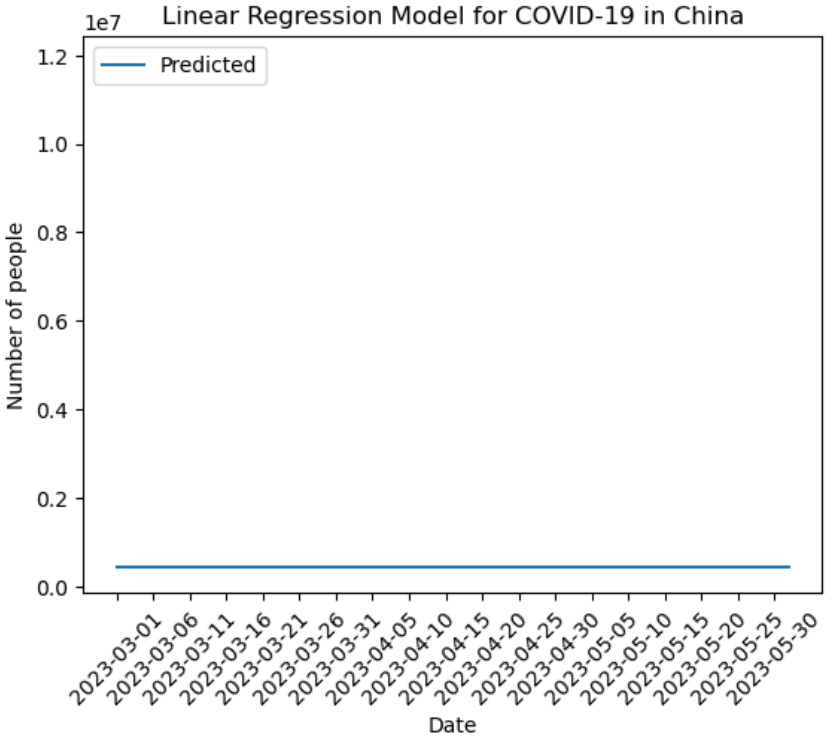

Figure 3. Linear Regression Model Prediction for COVID-19 in China.

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Figure 3 shows the number of COVID-19 infections predicted from March to June 2023 through machine learning and linear regression models. Since the units of the ordinate are large, the forecasted direction of the solid line looks almost constant. It can be seen from this figure that the number of new crown infections will remain at a low value in the future.

4.5. Compare forecasts

The Accuracy and R-squared values of Table 2 are very high, indicating that the prediction results of the model are very close to the actual results. Accuracy is a common performance indicator for classification models, and R-squared is a common performance indicator for regression models. The RMSE and MAE values of Table 3 are smaller, indicating that the prediction error of the model is smaller. Both RMSE and MAE have commonly used performance indicators of regression models, among which RMSE is more sensitive than MAE, because it squares the errors and then sums them, so the impact of larger errors on RMSE is more obvious. Furthermore, the predicted data and trends of the two graphs are very similar. are very slight drops and remain almost at the same value all the time.

Table 2. Prediction accuracy of the SIR model (Figure 2).

index	numerical value
MSE	1317.0000
Accuracy	0.9999
R-squared	0.9997

Table 3. Prediction accuracy of the ML regression model(Figure 3).

index	numerical value
RMSE	0.7831
MAE	0.7825

4.6. Discussion

It is very important to predict the number of COVID-19 infections in China in the coming months. This is because prediction can help us better formulate prevention and control measures and effectively control the spread of the epidemic. At the same time, prediction can also help us better arrange medical resources and reduce the impact of the epidemic on society and the economy.

In terms of drug procurement, predicting the number of COVID-19 infections in China in the coming months is of great significance for drug procurement. According to a study in international literature, predicting the development trend of the epidemic can help medical institutions prepare necessary drugs and medical equipment in advance to ensure the treatment and care of patients [13]. In China, a study pointed out that at the beginning of the epidemic, due to the limited drug procurement channels, some drugs were in short supply, which seriously affected the treatment of patients [14]. Therefore, forecasting the number of COVID-19 infections in China in the coming months can help medical institutions to formulate more scientific drug procurement plans, replenish inventory in time, and ensure the treatment needs of patients.

In terms of hospital bed management and distribution, predicting the number of COVID-19 infections in China in the coming months is also of great significance for hospital bed management. An international document pointed out that forecasting the trend of epidemic development can help medical institutions optimize the allocation and use of hospital beds, increase the utilization rate of hospital beds, alleviate shortages of medical resources [15]. In China, a study also pointed out that at the peak of the epidemic, the hospital bed management of medical institutions is very critical and plays a vital role in treating patients and controlling the epidemic [16]. Therefore, forecasting the number of COVID-19 infections in China in the coming months can help medical institutions to reasonably plan hospital bed

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resources, timely adjust the layout of wards and staffing, and improve the epidemic prevention and control capacity of medical institutions. In addition to the health sector.

In terms of logistics management, predicting the number of COVID-19 infections in China in the coming months can help logistics management. International literature pointed out that COVID-19 had a major impact on the logistics supply chain, including the shortage of raw materials and parts, the rise of logistics costs and the decline of logistics efficiency [17]. Therefore, forecasting the number of COVID-19 infections in China over the next few months can help logistics enterprises adjust their logistics plans in time, improve logistics efficiency and reduce costs. For example, when it is predicted that the epidemic will further intensify, logistics enterprises can take response measures in advance, such as increasing inventory, adjusting routes, etc., to ensure the timely delivery of materials.

In terms of corporate management, predicting the number of COVID-19 infections in China over the next few months is also of great significance for company management. International literature pointed out that COVID-19 had a profound impact on the company's operation and management, such as employee health, enterprise revenue, market demand, etc [18]. Therefore, forecasting the number of individuals infected with COVID-19 in China in the months ahead can help enterprises develop coping strategies, protect the health of employees, and stabilize revenue and market demand. For example, when it is predicted that the epidemic will worsen further, enterprises can make flexible work arrangements in advance, such as telecommuting, flexible working hours, etc., to ensure the health and safety of employees.

5. Conclusion

The problem of this paper is to predict the number of COVID-19 infections in China in the next four months. The research data comes from the official website of the World Health Organization. The models used in the study include the SIR model and the linear regression model. Statistical and predictive analysis of data through machine learning, modeling, and data visualization.

The main conclusion of this study is that the peak period of the first wave of COVID-19 infection has passed, and the number of COVID-19 infections in China will remain relatively low in the next four months.

However, this study also has some shortcomings: it does not consider too many external factors, such as holidays, residents' travel, weather changes, and so on. Moreover, the variation of COVID-19 was not fully considered. These issues can be predicted using a more powerful model by using more complex predictions, which can add and consider factors that affect the predicted data, thereby achieving more accurate data prediction; Find and collect more information about changes in a novel coronavirus, relevant policies and holiday arrangements to make the data more accurate.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230800

Analysis on the diet of basketball athletes

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Abstract. Food is essential for human survival. Dietary nutrients contribute significantly to our health. As a typical group that engage in a great deal of physical activity, athletes require a diet rich in the right kinds of nutrients in order to keep their bodies running at peak efficiency. Basketball is an intensely competitive sport. To achieve victory, both teams will engage in physical and technical confrontation. Contemporary basketball is distinguished by its intense rivalry. As basketball evolves and strives to reach a higher level, the physical requirements for athletes become increasingly stringent. Diet and nutrition are the foundation for athletes. A nutritious diet is a formula for success. Athletes should consume the appropriate amount of energy required by the body. This paper examines the physiological characteristics and nutritional requirements of athletes through a literature review. In-depth research will be conducted to determine the nutritional requirements of basketball players and make dietary recommendations. The purpose of this study is to make dietary recommendations for basketball players by analysing their fundamental signs and nutritional requirements.

Keywords: physiological characteristics, nutritional needs, basketball players, daily recipe.

1. Introduction

The Olympic Games centre on basketball, a physically hostile hand-centered activity. On December 21, 1891 [1], basketball was conceived by James Naismith, a physical education teacher at the YMCA training School in Springfield, Massachusetts. There will be four 10-minute quarters, and if the score is tied after the fourth, there will be one or more 5-minute halves to determine the winner.

Basketball is a very intense anaerobic activity that requires a lot of work from the lower body. Basketball players need agility, flexibility, and muscular endurance to be competitive on the court. Improving their efficiency requires monitoring and caring for their health. Proper nutrition is just as important as getting the right kind of training. A well-balanced diet can aid in the prevention of sports injuries, the speedy restoration of energy after strenuous exercise, the maintenance of peak athletic performance, the gradual overcoming of sports fatigue, and the successful resolution of the specific medical issues that arise during sports training.

Nikic et al. examined the physical make-up of 64 professional basketball players on the Greek national team [2]. Bioelectrical impedance was used to calculate the body fat and lean mass percentages of athletes across three age groups. Two-way analysis of variance was used to find out how age and position on the team affected body fat percentage, fat-free mass, and body mass index. Concurrently, Tsoufi et al. examined the nutritional intake and quality of a professional basketball team that received

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DOI: 10.54254/2753-8818/21/20230800

daily nutrition counselling during practise and game days and competed in a European level [3]. A 24-hour food diary was used to record daily dietary consumption, and from those data, a Healthy Eating index (HEI) was derived. The study found that professional basketball players had access to trained sports nutritionists on a daily basis, ate well most of the time, and ate as healthily as possible on game days. Maria used the general self-efficacy of 48 Polish basketball players to quantitatively measure their diet [4]. It is shown that the intake of particular nutrients has a direct bearing on the confidence levels of basketball players.

The first section of this study will focus on the physiological traits of athletes and the methods utilised to determine their physical parameters. Athletes have specific dietary needs that are determined by their workout routine and metabolic rate, and these needs are laid out in great detail here. Finally, the study offers advice on what kind of diet basketball players should follow before, during, and after games. This study provides a synopsis and framework for the existing literature. The physical traits of basketball players and their nutritional needs are outlined. As a result, readers will have a firm grasp on where the field now stands, be better equipped to spot relationships and contradictions in the literature, and have access to resources for conducting their own study into sports nutrition.

2. The physical characteristics of athletes and monitoring methods

During exercise, athletes experience a state of elevated stress, a sudden increase in energy consumption, a vigorous metabolism, and an increase in myocardial vascular capacity. These will result in strong muscle contraction, accumulation of metabolic byproducts, alterations to the internal environment of the body, decreased blood flow resistance, and increased sympathetic excitability. As an aerobic competitive sport, basketball will burn fat first. In addition to oxygen, it requires the participation of major muscle groups throughout the body, and the movement is lengthy and rhythmic. Therefore, basketball players must have a robust heart and a high pulse output in order to efficiently transport oxygen throughout the body.

In addition to comprehending the physiological characteristics of athletes, it is essential to monitor their physiological and physical parameters. In a review of the relevant literature, it is suggested that basketball players' heart rates be measured so that parameter variations during training can be analyzed. It involves monitoring exercise intensity, assessing player fatigue, and quantifying internal training burden. Finally, a personalized model -- SHRZ model -- is discovered by combining the players' responses during training with the parameters. It requires less time than other models and provides a useful balance of expertise, resources, and implementation [5].

3. Nutritional needs of athletes

3.1. Protein and lipids

Athletes' energy metabolism is affected by their individuality, level of fitness, and the length, intensity, and duration of their workouts. Athletes have specific dietary needs in order to keep their bodies in peak physical condition for training and competition. The breakdown of the body's three primary energy stores provides this fuel. The cost might be quite different from one form of physical activity to another. Sugar catabolism, followed by fat catabolism and protein catabolism, was the primary source of energy for all sports combined [6].

Protein is a crucial component of a healthy diet. Getting enough high-quality protein in your diet is essential. Urinary nitrogen loss and the load on the liver and kidneys both rise when protein intake is high. Proteins are essential for the maintenance and restoration of many different types of tissue. There is a connection between protein and the activation of muscles and the neurological system. It has been shown through scientific research that an athlete's urine nitrogen excretion will increase dramatically with an increase in activity intensity if protein is the sole nitrogen-containing food in their diet.

Fat is an essential part of a healthy diet since it provides a source of energy and is a cellular component. Among its many uses, it acts as a shock absorber, a source of energy, and a thermal insulator. In the diets of general athletes [7], lipids contribute between 25 and 30 percent of the total energy. Lipids

DOI: 10.54254/2753-8818/21/20230800

provide around half of the energy needed by the body during light and moderate activity. About 80% of the energy needed during prolonged exercise can be supplied by lipids. However, consuming an excessive amount of food reduces the body's ability to take in oxygen. Excessive fat also reduces fitness levels and raises the risk of cardiovascular disease. Athletes' endurance and post-workout recovery will be hindered if the ratio is too low. Basketball is an exhausting and time-consuming activity. Liver infiltration is prevalent because fat is involved in the metabolic process that produces energy. Therefore, basketball players should up their intake of fructose and anti-fatty liver meals like phospholipid while decreasing their intake of real fat. This helps the liver replenish glycogen stores after exercise by decreasing the amount of fatty liver infiltration.

3.2. Carbohydrates

Carbohydrates are also very important for athletes. Athletes rely on it heavily because of its ability to rapidly generate energy. Between 55 and 60 percent of an individual's calorie intake comes mostly from carbs. Basketball players benefit most from consuming carbohydrates before, during, and after practises and games. Also, it's the most important supplementable nutrient. A severe carbohydrate deficit not only has detrimental effects on basketball players' training quality and physical performance, but also disrupts the normal metabolism of other substances. Glycogen, derived from carbs, is the principal fuel source in basketball. Maintaining adequate levels of glycogen in the body helps prevent the breakdown of muscle and fat for energy, postpone the beginning of exhaustion, and protect the speed, stamina, endurance, and response time of athletes. The amount of glycogen in an athlete's body is directly related to his or her capacity for physical exertion.

3.3. Minerals and vitamins

The human body still needs minerals and vitamins, but only very little amounts. Minerals like iron and calcium are crucial for basketball players. Iron aids in the delivery of oxygen to working muscular tissue. Calcium helps maintain strong bones and is required for many bodily functions like muscular contraction and neuron activation. As a result, it's best to limit your intake of iron- and calcium-rich foods like liver, dark leafy greens, egg yolks, and dark fish. Although you lose water, minerals, and vitamins through sweat during the first 30 minutes of activity, you are not required to rehydrate at this point. A water supplement is meant to restore the fluids lost via sweat and keep the body from becoming dehydrated. Low-sugar, low-sodium water with inorganic salts is recommended. Water loss can be substantial during a basketball game because the game is typically played inside without air conditioning. A significant amount of fluid will be lost through sweat even in chilly settings. In order to estimate how much water to drink during training, games, and recovery, athletes can calculate their individual perspiration rates as an alternative to depending on perceived sweat rates or thirst. A professional basketball player's daily needs, expressed in terms of kilogrammes of body weight, are detailed in TAable 1.

Table 1. The daily requirement per kilogram of body weight for a basketball player [8].

Energy 55-60kcal	Carbohydrates 8.5-10g	Protein 1.0-1.2g
Minerals	Potassium	3-4g/d
	Calcium	1000-1500mg/d
	Iron	20-25mg/d
	Zinc	20-25mg/d
Vitamins	Va1	500μg
	Vb1	3-5mg
	Vb2	2-2.5mg
	Vc	140mg
	Ve	30mg

DOI: 10.54254/2753-8818/21/20230800

4. An athlete's diet—A basketball player

Maintaining a healthy, well-rounded diet is crucial. High-carb, high-energy, and high-nutrient-density diets are all options for athletes who want to optimise their performance in their chosen sports.

Basketball players need to be physically active, energetic, and healthy, all of which are supported by eating habits that emphasise health and wellness. Many basketball players have high energy needs because of their height, the intensity of their training, and the fact that some young athletes are still growing and developing. Basketball players treat and recover from a variety of injuries throughout practice, games, and postgame. Thus, there are three unique periods of professional basketball players' diets.

Basketball players need to make sure they're feeling good physically and mentally before training or games. Typically, three to four hours before a basketball game, the players eat dinner. This meal is very important since it provides fuel for the forthcoming race in the form of carbohydrates and fluids. Additionally, eating enough protein before competitions can keep you from getting hungry. Moderately replenish with carbohydrate-rich foods like muesli, milk, fruit, etc., during the marathon. Rehydrating, especially with electrolyte-rich sports drinks, should be a priority for the duration of the race. They help replenish your glycogen and electrolytes. Athletes must need downtime to recover. An athlete's post-workout meal should consist primarily of carbs, with some protein and plenty of water and electrolytes. Restorating muscle glycogen levels will guarantee that glucose will be stored. In order to restore muscle growth and function, protein supplements are needed. As can be seen in Table 2, rehydration serves to replace fluids that were lost throughout the competition.

Table 2. The diet of basketball players [9].

Pre-game meal	Pre-game snack	Recovery food
Wrap or sandwich with chicken and salad	Yoghurt with fruit salad	Chicken, avocado and salad sandwich
Muesli with yoghurt and berries	Banana and a handful of almonds	Salmon with brown rice and steamed vegetables
Soup served with toast	Peanut butter on rice cakes	Dairy-based fruit smoothie
Pasta with tomato-based sauce	Toast with vegemite	Yoghurt + muesli with nuts and seeds
Chicken stir-fry with rice	Fruit smoothie	Burritos with beef, cheese, avocado and salad

5. Conclusion

The overall physical quality of basketball players is rising as the sport gains popularity around the world. The success of the athletes depends on their ability to maintain a healthy nutritional balance. Adequate and acceptable nutrition may not enhance athletes' performance in competition, but it can help them perform at a normal level [10]. Basketball players have unique nutritional requirements in comparison to those in other sports. A reasonable nutritional supplement can not only keep athletes in peak physical and mental condition, but also help them compete for a longer period of time. This study begins with a brief overview of basketball players' physiology, and then proceeds to an analysis of athletes' dietary requirements by integrating these parameters with their training and performance schedules. Basketball players have different dietary needs before, during, and after games. Therefore, various food suggestions are provided for each of the three phases. There are, however, downsides to this content. This paper primarily consists of a review of related works. There hasn't been enough research done on basketball players' diets and body mass indexes. While they have a common bond as basketball players, each player also has their own distinct personality. The universality of the paper's findings and

recommendations is not proven; more research and practice in the field will be required to confirm its applicability to all basketball players.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230801

Gastric cancer stem cell: Carcinogenesis and targeted personalized therapies of cancer stem cell

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Abstract. Gastric cancer (GC) is one of the most common cancers in worldwide range which ranks fifth, and it is also third most common cancerous death reason. Although there has been a decline in the rate of incidence and mortality over recent fifty years, cure for GC is extremely hard to achieve. The symptoms are not conspicuous at early stage of the GC patients usually, which is the reason GC is diagnosed at terminal stage. And it is worse that the prognosis effect is outrageously bad, the median survival times are mostly less than a year. Under this condition, prevention is the most efficient method to reduce the incidence and mortality, meanwhile, the application of newly emerged therapy should be attached importance to GC. To create accurate treatment to achieve this goal, it is necessary to comprehend the mechanism of the carcinogenesis, and mechanism of cancer development, the critical transformation points derived from those mechanism will divide the different methodology applying period. This article mainly focuses on the cancer stem cell (CSC) aspect of carcinogenesis, cancer development to inform the points which can be utilized to intervene and new therapy method.

Keyword: gastric cancer, carcinogenesis, cancer stem cell (CSC), targeted personalized therapy.

1. Introduction

1.1. Introduction of cancer stem cell model

The cancer stem cell model declares that only some subgroups of cancerous cells dominate the formation, self-reproduction, and differentiation of tumor, while the daughter cells only has restricted proliferate ability because epigenetic regulation occurred [1]. It is analogous to the structure of normal human tissue including the stem cell responsible for differentiation and other heterogeneous cells, however, those cells are not in order like normal organ [2]. This model forecast an inspiring future for eliminate the tumor without killing all the cancerous cells like radiotherapy and chemotherapy by removing the cancer stem cells (CSCs) specifically. Unlike traditional therapy will kill normal cells simultaneously if the targeted cells can be identified precisely, CSC targeted cell therapy can reduce the developing potential with much less harm. However, the features to determine the CSC are key difficulty in target therapy. The biomarks found in gastric CSC do not have enough specifications to distinguish them from normal stem cells [3]. Other methods like side population methods are also highly valid. The reasons why it is intricate to specialize the CSC are mainly caused by the plasticity of CSC. The silence of CSC properties

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DOI: 10.54254/2753-8818/21/20230801

is reversible during the process of the traditional therapy like radiation [4]. Hence, it is necessary to be cautious to combine radiation therapy and targeted therapy.

1.2. Introduction of the gastric carcinogenesis

In GC, there are long-term and multiple-step processes called correa's cascade including metaplasia and dysplasia caused by atrophic gastritis, then, gastric cancer [5], meanwhile, surgery is still primary treatment of cancer stage and complete cut is the sole operation to achieve totally recover [6], the manifestation of the patients after treatment is not satisfying, high mortality mainly aroused by metastasis, Chemotherapy resistance [7, 8]. In this condition, precautionary measurements are vital to decrease the morbidity of GC in the present stage to stop the progression of the tumor formation. To attain this objective, the process of the whole gastric carcinogenesis should be evaluated.

1.3. Metaplasia

It is a common lesion during the formation of tumor, and highly related to the intestinal type GC, another type is diffuse type which is more determined by inheritance [9,10]. *Helicobacter pylori* genomics are considered as the main factor to trigger correa's cascade, host genetic factors ABO blood type, genetic predisposition, environmental influence, diet, and intestinal microbiota are other causes [11].

As consequence of the infection of *Helicobacter pylori*, gastritis happens, and if it is atrophic it will cause the loss of glandular oxyntic mucosa due to long-range inflammation [12]. Finally, the gastric epithelial cells act like intestinal phenotype cells, containing eosinophilic enterocytes with a distinct brush border (apical microvilli to promote digestion) and goblet cells formed sufficiently [13]. Some research had indicated that the intestinal metaplasia spread is related to the gastric crypts [14].

In this carcinogenesis stage, the personalized targeted treatments are under exploration for diagnosis, treatment, and the goal of prognosis. To establish the method of personalized treatments and measurement of potential of evolution to invasive carcinoma, the biomarkers and the molecule pathway should be illustrated. Novel diagnostic could expound polymorphisms in genes, alteration in the expression of miRNAs and lncRNAs, as well as microbiome happened in this stage [15], meanwhile, the corresponding transcription factor (CDX-1 protein), telomere reduction, microsatellite instability, and mutations in p53, APC, and K-Ras have been identified in Intestinal Metaplasia [16]. More clinic-related research and specific molecule pathways on those biomarkers could pave the way to stop carcinogenesis here.

1.4. Dysplasia

The dysplasia of gastric epithelial cells are considered as the portent of carcinoma, histologically evident neoplastic epithelium without tissue invasion, which are developed under the effect of atrophic gastritis and metaplasia [11,17]. In clinic research, the classification is quite complicated like Padova methodology placing emphasis on clinical application. Basically, the morphology of the dysplasia cells are characterized by neoplastic epithelium phenotype cells which are restricted to glandular structures inside the basilar membrane. Compared to metaplasia phase, the metaplastic glandular structures are not orderly—irregular shape equipped with thick membrane, mucus secretion shrinkage even shutoff, moreover, the nuclei of those cells are pseudo layered with evident amphophilic nucleoli [18].

Currently, there is evidence to indicate that the malignant tumor potential of the high- grade gastric dysplasia can reach 10% to100% [19]. Surgery (endoscopic mucosal resection), nowadays, this physical operation is the only way to restrain the carcinogenic progression on high- grade gastric dysplasia, meanwhile, endoscopic ultrasound and adequate sampling provided can ensure submucosal invasion is excluded [19]. Presently, the comprehensive system active mechanism of how the dysplastic epithelium evolve to invasive carcinoma, how the mutated cells expand to surrounding environment doesn't construct, consequently, the treatment scheme after endoscopic mucosal resection is still under exploration, that remains a hard problem to solve, always a risk of occurrence of synchronous or metachronous gastric neoplasms in other sites [20].

DOI: 10.54254/2753-8818/21/20230801

The available scientific literature suggests that the eradication of H. pylori infection results in a modest deceleration of carcinogenesis. However, it is important to note that all available clinical trials have been conducted in adult subjects who were in advanced stages of atrophy and intestinal metaplasia, likely infected for a period of five or more decades. Nonetheless, recent research has postulated that the inflammatory process caused by H. pylori may lead to oxidative damage, which could contribute to neoplastic progression. It is plausible that oxidative insult may have been present in the gastric mucosa for a considerable period before the initiation of anti-Helicobacter treatment. Consequently, molecular events that eventually lead to neoplasia may have reached a point of irreversible transformation. [21].

Recently, the pathway of the gastric CSC formation from dysplastic epithelium has been discovered gradually, a step towards a comprehensive system active evolution pathway. This had stepped forward in discovering the origin of the gastric cancer stem cells, researchers found that the dysplastic stem cell (DSC) populations CD44v6neg/CD133/CD166 (DP) could be one target of CSCs ancestor. The dysplastic cell lineages are maintained and differentiate through a Wnt ligand-independent signaling pathway, mediated by CK1α/β-catenin. Xenograft studies demonstrated that the DP-DSCs clonally evolve towards multiple types of gastric adenocarcinomas and promote cancer cell heterogeneity by acquiring additional genetic mutations and recruiting the tumor microenvironment [22].

1.5. Invasive Carcinoma

Gastric cancer comes to mature tumor stage when invasive carcinoma is formed. The Lauren classification is widely accepted in this phase, what divided the invasive carcinoma into intestinal and diffuse types on account of the glandular structure [23]. In correa's cascade, the progressively increasing genetic and epigenetic alterations accumulated [24]. While clinical traits and gene alterations of the intestinal and diffuse GC types are different [23].

In intestinal tumors, tumor cells usually stick together, formed in tubular or glandular shape. It is common that this type is related to lymphatic or vascular invasion. Notably intestinal cancer has a better prognosis [25].

In diffuse gastric cancer, there exists more complicated problem to deal with, the tumor cells act more solitude behavior by lacking adhesion, representing as single cells or smaller group compared to intestinal type, and act as single cells or small subgroups dispersed among stomach. Peritoneal metastasis can be discovered in diffuse gastric cancer, otherwise, precursor lesions are hard to detect in diffuse type cancer [26].

It is immensely significant to illustrate molecule pathway of carcinogenesis, mutated gene expression products, those knowledge contributes to direction of personalized therapies to restrain the process of carcinogenesis, targeted pharmaceutical to eliminate CSCs, and clarification of clinical metastatic potential, prognosis, resistance to chemotherapeutic agents. One the other word, the abnormal genes and genetic information expression pathway could be the targeted for personalized therapy if the products are related to carcinogenesis.

By application of modern technique like microarrays and comparative genomic hybridization, the genetic anomaly incidents of chromosomes can be detected in gastric cancer, and the Wnt, TGF or E-cadherin signals had been approved the vital roles in carcinogenesis [27].

Genes deregulations are elaborated progressively as well. In intestinal gastric cancer, Microsatellite instability (MSI), mutation of KRAS and APC, and ERBB2 exaggeration, MLH1, MGMT, and CDKN2A genes silenced caused by CpG island hypermethylation are frequently found in intestinal type GC [23]. However, loss of heterozygosity at chromosome 17p (p53) and mutation or loss of E-cadherin are more often detected in the development of diffuse-type gastric cancer, and, metastasis of gastric tumor cells usually be regarded as the blame of loss of p27 and gene amplification of K-sam and c-met genes [28]. Those mutated genes are the origin of the differentiation of CSCs, and proliferation, adhesion, and migration of tumor cells, the ideal targeted point may hide in them.

2. Discussion

On account of the high relevance between H pylori infection and gastric carcinogenesis, scientists want to figure out the effect of reducing the H pylori infection, however, this operation just shows limited impediment of carcinogenesis, the possible trigger is the long-term oxidative damages induced by the inflammatory process have already generated sufficient molecule incidents to initiate correa's cascade [29].

Presently, primary therapeutic treatments in invasive tumors are still cases surgery, chemotherapies and radiotherapies, nevertheless, worldwide the 5-year survival rate remains at 25%, which is a unsatisfactory figure [30]. And only a marginal survival benefit caused by chemotherapy analyzed by meta-analyses of random trials [31]. It is relieved that, this situation can be illustrated by cancer stem cell theory, while the targeted therapy on cancerous stem cells may have brightness on successful cure. The principle of those chemotherapies and radiotherapies is inflicting DNA damage, then to trigger senescence in cancer cells, a method called therapy-induced senescence (TIS) resulting in lessening tumor size and accumulated immune cells such as neutrophils, monocytes as well as T-cells [32-34]. But the incompleteness of elimination of tumor cells remain potential trouble, those tumor cells what escape from TIS gains extra mutation to get stemness and evade senescence over a long-term course of TIS to rejuvenate tumor, besides, chemicals secretion of senescent cells always represent tumor-promoting factors [35,36].

Furthermore, the reasons why chemotherapy does not act as an excellent tool to cure gastric cancer are consistent with the modified model of the CSC theory, dynamic model, differentiated cell populations have the potential to reverse to CSC governed by tumor cell environment, specifically, governed by inducing factors produced by stromal cells [37].

Focus on the environment CSCs located, which are surrounded by a sheet of subepithelial myofibroblasts (SMFs), this niche is the source of growth and differentiation factors, in recent research, the fact that CSC could activate stromal fibroblasts (SFs) and become myofibroblasts had been shown, those transformed cells could emit vascular endothelial growth factor A (VEGFA) and other angiogenic factors, a promising preventing strategy inspired by this mechanism by restraining tumor cell-derived factors [38]. Understanding the origin of CSCs and their interaction with niches would be helpful for precisely targeting CSCs.

3. Conclusion

Those findings of molecule pathways of carcinogenesis, altered genetic information, and abnormal gene expression products construct the foundations for personalized treatment strategies. The targets of chemical pharmaceuticals and immunotherapy like the Car T cells method to stop carcinogenesis, reduce CSCs, and restrict metastasis are revealed while discovering the molecule pathway of carcinogenesis.

With the development of unambiguous mechanisms, findings of biomarkers, and the microenvironment of CSC, more options for scientists emerge to design experiments.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230803

Effects of dietary structure on obesity and following diseases

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Abstract. Due to the quite unhealthy diet that people normally have in recent years, obesity becomes a more and more popular health issue. In recent years, it even becomes a risk factor for deaths. This essay mainly focuses on how the components in diet may cause obesity and other diseases and what components are required to make up of a healthy and balanced diet. To figure out a possible solution for the raising negative consequences brought by obesity, like coronary heart disease and type II diabetes, some investigations have been done. The researchers provided mice with either a diet high in fat or a normal diet to simulate the healthy diet and a quite unhealthy one that humans may take. Some extra tests like glucose tests are taken to show whether the mice have got the symptoms of diabetes. The simulation lasted for two months, an overall higher weight for the mice that were fed with high-fat diet was seen, which indicates that high-fat diet will put more weight on creatures. Also, high-fat diet overall leads to a higher glucose level, which suggests that unhealthy diet may show a decrease in the ability to decompose glucose and therefore causes diabetes. After the data is obtained and the result was drawn, nutritionists may cooperate with doctors and try to figure out the best possible diet for the patients that have very high risk of getting or already suffering from obesity, CHD or type 2 diabetes.

Keywords: diet, coronary heart disease, obesity, type II diabetes.

1. Introduction

Obesity has become one of the major health problems that the world needs to concern. It already has been spanned from rich countries to regions of all income levels [1]. The ranking of obesity in the number of deaths by risk factor increases and has been one of the TOP 5 factors that causes death the most as shown in Figure 1-2.

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DOI: 10.54254/2753-8818/21/20230803

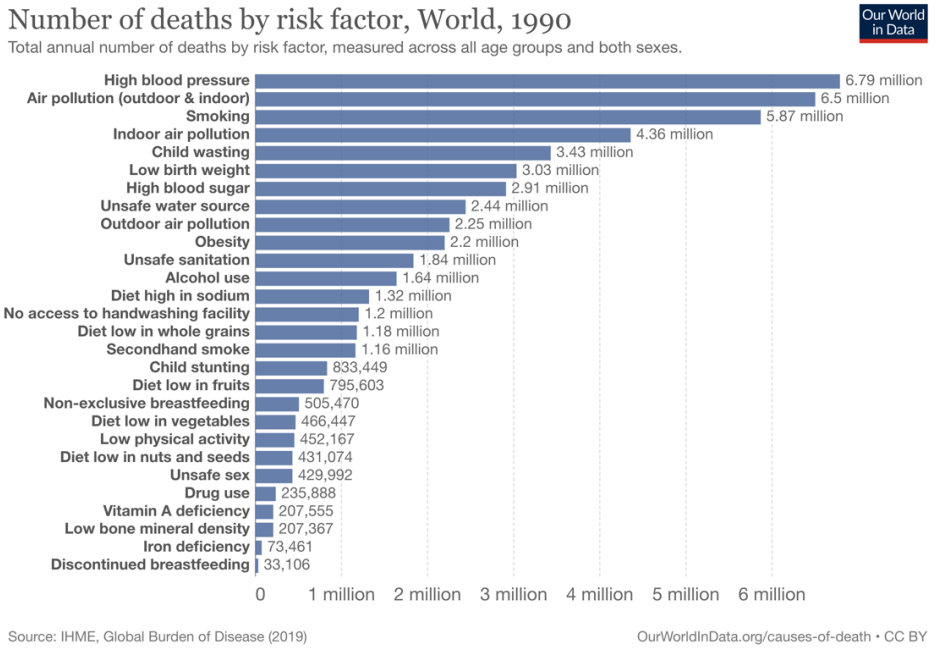

Figure 1. Ranking of the highest risk factors of deaths in the world in 1990 [1].

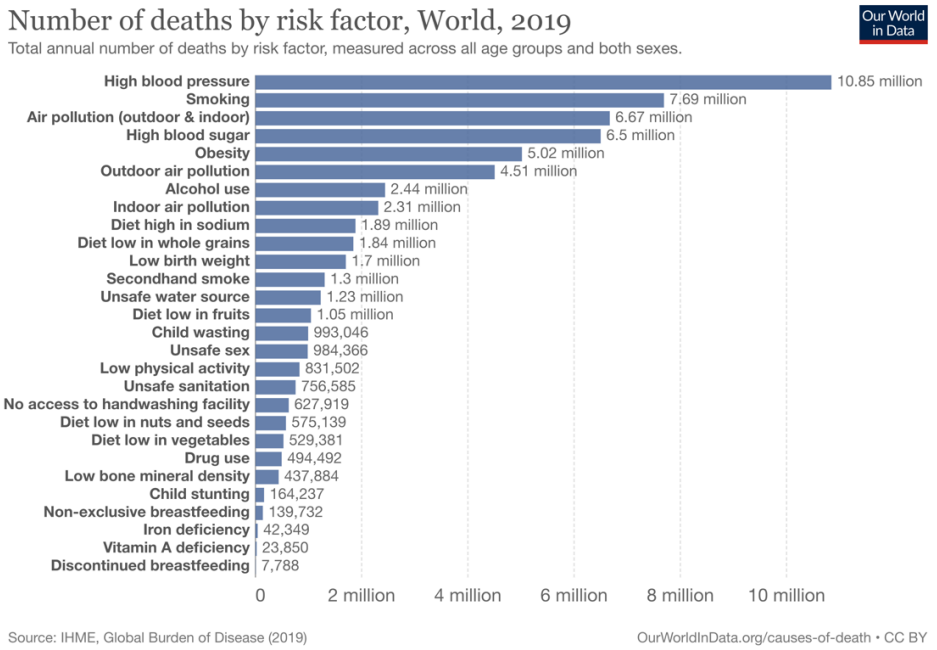

Figure 2. Ranking of the highest risk factors of deaths in the world in 2019 [1].

Additionally, obesity is very likely to cause or co-exist with other diseases like CHD, high blood pressure, type II diabetes etc. These will do great harm to people's health, but human beings should still try hard to figure out methods to weaken the threats brought by these diseases. Controlling a daily diet can be a possible method to minimize the negative effects of obesity and those diseases.

Diet in humans often refers to the types of food and drinks that a human being regularly takes in. A well-balanced diet is essential for keeping good health conditions as well as preventing the spread of chronic diseases. Diet structure is the composition of a person's daily diet, including the amount and different types of micronutrients and macronutrients consumed. Micronutrients are minerals and

DOI: 10.54254/2753-8818/21/20230803

vitamins, while macro nutrients include carbohydrates, fats and proteins [2]. The case is analyzed in the main body mainly focuses on the effect that high-fat diet may bring to creatures and the potential risks it may have by doing a simulated experiment first on mice [3]. The researchers use various tests to show whether the test-taker has got the disease or not. For example, by doing glucose test before and after the diet control on both groups of mice, researchers may figure out whether the mice got the diabetes or not. Plus, this experiment provides solid evidence to show that having high-fat diet for a long term is very likely to cause an overall increase in body weight and may further develop to obesity. Currently, researchers have already figure out the probable causes of diseases like obesity, coronary heart disease and diabetes, which all may contribute to an unhealthy diet. Therefore, the importance of controlling the dietary structure and having a healthy one is highlighted. However, scientists only have figured out a quite broad healthy diet plan like telling people what to eat and what is not recommended to eat. Although they are trying hard to deliver the correct knowledge on eating healthily, the diet they provide are usually not quite acceptable to the majority of people in the public. It is quite hard for them to get rid of the addictive unhealthy diet and convert to this eating plan. As a result, researchers still need to pay more efforts on figuring out a diet plan that fits people's requirement of not only healthy, but also tasty, which may be more acceptable to them.

The motivation for author to write this review is mainly because author's family members are suffering from coronary heart diseases and type II diabetes and author had noticed the unhealthy eating habits that they had before. After author's family members got the doctor's advice, they started to make some changes on their diet. Admittedly, some positive results were seen, but not for long. Therefore, the author wants to figure out what type of diet can truly prevent or reduce the risk of getting these diseases and the diet structure that is beneficial to help those patients recover. The following research will first focus on the case description and analysis mentioned before, explain why keeping a healthy diet is essential and figuring out the components in the diet will cause these diseases.

2. Main body

2.1. Case description

From this study, the researchers want to emphasize the importance of maintaining a normal diet to reduce the risk of getting a stroke, especially when the patient already has or has a high risk of type II diabetes. The significance of carrying out this study is that stroke will probably lead to disabilities in the long-term and even death, while the several major risk factors of stroke include diabetes, poor quality of diet, and obesity. In industrialized countries, the rate of obesity has sharply increased and reached an epidermic level. The study aims to figure out whether a high-fat diet (HFD) would have a more severe negative impact on stroke because of type II diabetes, the researchers use mice to create a model for the result of this experiment. For the control group, the mice are fed on a normal diet (ND), while the experiment group has HFD instead. Both are fed on the corresponding diet for 2 months. Pre-diabetic status can be simulated and assessed through Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT). From the experiment, the researchers concluded that after taking HFD for 2 months, the situation of hyperlipidemia and high blood glucose in adult mice can be more severe. The researchers used several graphs to interpret the data they obtained from two groups of mice (Figure 3-5) [3].

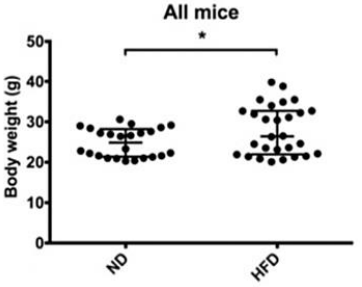

Figure 3. Body weight of all mice, Range of HFD overall has a higher value than range of ND [3].

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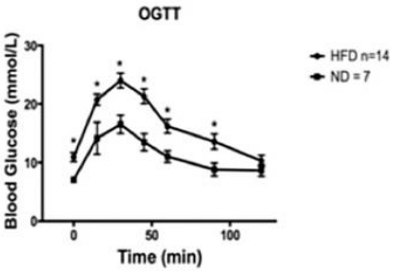

Figure 4. Results of OGTT, HFD has higher blood glucose level than ND [3].

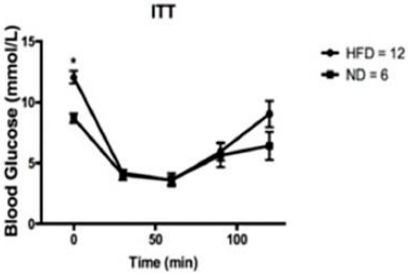

Figure 5. Results of ITT for both groups of mice, No significant difference in trend [3].

2.2. Analysis

2.2.1. Nutrients required for every day's diet. There are various nutrients required to be taken in to meet the need of the body and also believed to have impacts on all physiopathology processes [4], which includes carbohydrates, proteins, fats, vitamins, and minerals. But the amount of intake for each nutrient varies among people as each person is of different age, sex, activity level and other factors related to health [5]. The World Health Organization has emphasized the importance of having a balanced diet, they advocate a diet rich in foods from all food categories to ensure that body obtains all of the nutrients it requires for optimum health [6]. The Dietary Guidelines for Americans also promote a nutrient-dense eating pattern that contains all fruits, whole grains, vegetables, lean proteins, and healthy fats [7]. To decrease the risk of these chronic diseases, it highlights the significance of reducing the intake of added sugar, saturated and trans fats, and salt. Studies have also shown that the quality of the diet, rather than just the quantity of nutrients consumed, is crucial for optimal health. For example, research discovered that a diet rich in whole plant foods as mentioned above, has been related to a lower risk of chronic diseases [8]. Another study suggested that eating a Mediterranean-style diet that is rich in those nutrients mentioned above, was connected with a decreased probability of getting heart disease [9].

2.2.2. Components that will cause disease by overtaking it. Reduced consumption of saturated and trans fats has been demonstrated in studies to significantly lower the risk of CHD. In the Trail of WHI's Dietary Modification, women were randomly allocated to one of the two types of diet, a low-fat diet or a typical diet. After eight years, the low-fat diet was related with a substantial decrease in the incidence of CHD [10]. In addition to limiting saturated and trans fats, boosting unsaturated fat consumption has been demonstrated to lower the risk of CHD. Unsaturated fats, which may be found in foods such as nuts and fatty fish, have been demonstrated to lower the LDL cholesterol, while an increase in high-density lipoprotein (HDL) cholesterol level is seen as well, popularly known as "good" cholesterol, in the blood.

Fiber is another dietary factor that has been related to a decreased risk of CHD. Fiber, included in wholegrains, vegetables and fruits, has been shown to lower cholesterol levels and inflammation, both of which are risk factors for CHD. A 10-gram increase in dietary fiber intake per day will lead to a 14% reduction in the risk of CHD in a meta-analysis of 22 prospective cohort investigations. Type II diabetes develops when the body develops resistance to the hormone insulin, which regulates blood sugar levels.

DOI: 10.54254/2753-8818/21/20230803

This causes an excessive amount of glucose exists in the blood, which can lead to bunch of health problems [11-12].

One of the most important dietary factors that contribute to type II diabetes is the intake of added sugars. Added sugars, which are found in many processed foods and beverages, have been shown to raise the risk of type II diabetes by causing insulin resistance and inflammation. A high intake of sugar-sweetened beverages was related with a 26% increased risk of type II diabetes in a meta-analysis of 11 prospective cohort investigations. In addition to added sugars, refined carbohydrates consumption has also been related to an increased risk of type II diabetes. Refined carbohydrates, such as white bread, rice, and pasta, are rapidly digested and absorbed, causing a surge in blood sugar levels. This can result in insulin resistance and the onset of type II diabetes. On the other hand, taking in more whole grains has been proved to lower the risk of type II diabetes. Whole grains, which include brown rice, oatmeal, and whole wheat bread, contain fiber and other nutrients that aid with blood sugar regulation and insulin sensitivity [13].

2.2.3. Importance of adjusting diet structure. Dietary structure is critical in the development of chronic diseases, including CHD and type II diabetes. Both diseases are highly associated with poor dietary habits, particularly those diets high in both saturated and unsaturated trans fats, processed carbohydrates, and added sugars. The author will highlight the impacts of dietary structure on CHD and type II diabetes, as well as suggestions for altering dietary habits to lower the possibility of developing these disorders. Coronary heart disease (CHD) is defined by plaque formation in the arteries where blood is supposed to be delivered to the heart. This can cause artery constriction, limiting blood flow to the heart and raising the risk of heart attack or stroke. While several variables contribute to the development of CHD, dietary structure is a major modifiable risk factor. High saturated and trans fats dietary habits have been firmly linked to an elevated risk of CHD. Saturated fats are typically present in animal products such as meat and eggs, as well as plant-based oils such as coconut oil. Trans fats, which are produced by a process known as hydrogenation, can be found in a wide variety of processed foods, including margarine, baked and fried goods. These fats can increase cholesterol levels in the blood, leading to the formation of plaque in the arteries.

Diets high in unsaturated fats, particularly polyunsaturated and monounsaturated fats, on the other hand, have been found to lower the risk of CHD. These fats may be found in nuts, seeds, avocados, and fatty seafood like salmon and tuna. They can aid in cholesterol reduction and general heart health. Consumption of refined carbohydrates and added sugars is another dietary component significantly associated to CHD. White bread, spaghetti, and rice are examples, as well as sugary beverages and sweets. These foods can cause blood sugar surges, which can damage the artery walls and lead to plaque formation. Furthermore, diets heavy in refined carbs and added sugars can cause weight gain and obesity, both of which are risk factors for CHD. To lower the risk of CHD, it is crucial to follow a diet rich in nutrients mentioned before, while minimizing intake of saturated and trans fats, refined carbohydrates, and added sugars. The Mediterranean diet, which stresses whole foods and healthy fats, has been demonstrated to be very helpful in lowering the risk of coronary artery disease. Other measures for lowering the risk of CHD include keeping a healthy weight, getting regular exercise, and not smoking.

Type II diabetes is defined by raised blood sugar levels as a result of the body's inability to manufacture or use insulin efficiently. This can result in a variety of consequences, including nerve damage, renal disease, and heart disease. Dietary structure, like coronary artery disease, is a key modifiable risk factor for type II diabetes. High-refined-carbohydrate and added-sugar diets have been firmly associated to an elevated risk of type II diabetes. These foods can cause blood sugar increases, which can lead to insulin resistance, a major precursor to type II diabetes. Furthermore, high-fat diets can lead to gaining weight and becoming obese, both of which are risk factors for type II diabetes. Diets rich in fiber, particularly whole grains, fruits, and vegetables, on the other hand, have been demonstrated to lower the incidence of type II diabetes. Fiber can help control blood sugar levels and enhance insulin sensitivity, lowering the risk of developing type II diabetes [13-14].

DOI: 10.54254/2753-8818/21/20230803

3. Conclusion

High-fat diet and unhealthy diet structure can truly bring health issues like obesity, CHD and type 2 diabetes. According to the experiment done by researchers, if mice take a high-fat diet for a quite long term, they will show a gain in body weight as well as a lack of the ability to digest glucose to some extent. Simulate that into the condition of human beings, as the diseases are caused by diet, some scientists believe that the symptoms of these diseases may be reduced by adjusting dietary structure. Although what should be included in a daily diet has already been figured out, it is hard for most people in the society to determine for how much of each component that they may take for a daily diet, the balanced diet is always hard to reach. The importance of adjusting diet to minimize the negative effects that those diseases brought to human beings are also emphasized and proved how the overtaking of some components that people must have in daily diets, like fats and fibers, may lead to a result of increased risks for obesity and CHD in some journals. Because of the significance of adapting to a balanced diet and understanding of the vital nutrients that the human body demands, it provides solid and useful data and background for further investigating a more specific and detailed diet which is much easier for people to follow and therefore generally change their diet structure to a healthier and balanced one. The limitation of this review is that the research is done on mice, there might be some deviations when it is adapted to the situation of human beings. Also, the suggestions provided for adjustment of dietary structure are not that in detailed enough to directly conclude a specific diet plan that can be recommended to all people in the public. The author hopes that more researches and investigations could be done that can help nutritionists to figure out a much more detailed diet plan that can be shared with the whole public and therefore the new diet plan may be able to lower the risk of leading to death because of diseases like obesity, CHD and type 2 diabetes.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230804

Applications of the CRISPR-Cas9 system in cancer models

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Abstract. Cancer has a high mortality and prevalence rate in the world. CRISPR-Cas9 is one of the novel and most common gene-editing techniques. Compared with the first two generations of gene-editing technologies, CRISPR-Cas9 system has the advantages of easy design, low cost, high efficiency and so on. sgRNA guides Cas9 to the site of the targeted gene, and Cas9 cuts the DNA strand at that site, triggering the NHEJ or HDR mechanism so as to achieve the purpose of deletion or insertion. CRISPR-Cas9 can be combined with other factors for other purposes, such as CRISPRa, CRISPRi, and base editing. The CRISPR system now has been used extensively for research into biological mechanisms and disease treatments. Since cancer is controlled by genes, a number of researchers in recent years have looked at using the CRISPR system to treat cancer. The CRISPR technology has greatly improved our understanding of cancer and the factors that affect it, and has had a major impact on the study and treatment of cancer. CRISPR gene editing can quickly and efficiently generate gene knockouts and regulate gene expression to identify relevant genes that influence cancer growth. This review systematically introduces CRISPR-Cas9 and its application methods, delivery modes, and discusses some studies using cell lines and organoids in vitro and animal models for cancer therapy in vivo.

Keywords: CRISPR-Cas9, cancer, therapy, model.

1. Introduction

Cancer is a common type of malignant tumor derived from epithelial tissue. Cancer cells devour healthy cells and evade the immune system, tricking it into helping them survive and grow. Cancer cells cause organ failure by pressing, squeezing, consuming, or destroying organs, which leads to abnormal functioning and mechanisms in the human body. Cancer can occur in almost any part of the body. There are cancerous tumors called malignant and not-cancerous tumors called benign. Cancer can diffuse to a new part of the body to achieve metastasis, which is a transfer of position via the lymphatic system or blood flow.

Common clinical manifestations of cancer include masses, pain, ulcers, bleeding, obstruction, and some neurological symptoms. There are many factors affecting the etiology of cancer, including exogenous factors such as lifestyle, environment, biological factors, irritations, and trauma, as well as endogenous factors such as endocrine factors and most genetic factors, which is the least common factor. The World Health Organization published a 2020 report on the cancer population in both sexes and all ages. In Asia, the death rate from cancer is 58.3 percent [1]. Clustering regular interspersed short palindromic repeats (CRISPR) is a molecular biology technique. CRISPR-Cas9 is based on an acquired prokaryotic immune system that can edit genes in cells and organisms. In this system,cas9 protein,

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DOI: 10.54254/2753-8818/21/20230804

sgRNA and other components are very important. There are many variants of Cas9, each type has its own set of characteristics and sequence recognition criteria, providing more flexibility to adapt as a research or therapeutic tool. As one of the most popular gene editing techniques, the CRISPR-Cas9 system can edit, activate, and inhibit the targeted gene. CRISPR-Cas9 is now used extensively for research into biological systems and treatments for human diseases [2]. This review offers an expanded description of the applications of the CRISPR-Cas9 system in cancer models.

2. CRISPR-Cas9 system

CRISPR is a kind of natural immune system of prokaryotes with a highly repetitive genetic sequence that currently protects them from foreign DNA and phages. When some bacteria were invaded by a virus, they were able to integrate a small piece of viral gene into the crisper spacer region. The crRNA precursor (pre-crRNA) is modified and processed to form a guide RNA (gRNA). When the virus strikes again, the bacteria can use this CRISPR system's gRNA for recognition by base pairing and target the Cas protein to viral DNA and cut it, rendering it useless [3]. By comparing with the first two generations of genome editing techniques, such as ZFN and TALENS, the CRISPR-Cas9 system may have a higher success rate and lower cytotoxicity. It may cost less due to the easy design and amenities for high-throughput screens. The CRISPR-Cas9 system also allows editing to occur simultaneously on different gene target sites. However, CRISPR-Cas9 technology has a lower specificity (22 bp) compared to ZFN, which has a target site length of 18–36 bp, and TALENs, which has a much longer target site length of 30–40 bp.

The CRISPR-Cas9 system is a genome engineering technology that can be used in many models to change the sequence or expression of genes. This gRNA consists of a CRISPR RNA (crRNA) sequence that binds to the target site and a tracRNA that binds to the Cas9 protein. The guide sequence in gRNA has 20 bp. The combination of crRNA and tracRNA formed a single guide RNA (sgRNA) [4]. The Cas9 protein will be guided by the sgRNA, which has the sequence of the target region of the gene, to bind to the target site. The double-strand break created by Cas9 nuclease causes a frameshift in the nucleotide sequence, inactivating the specific protein. Cas proteins require short sequence motifs, protospacer adjacent motifs (PAM sequence), to identify target sites [5]. After the Cas9-sgRNA complex combines with one of the DNA strand by identifying the PAM sequence on the complementary strand, the surrounding DNA is pulled apart to form two single-stranded DNA strands in an R-loop. The complementary strand will be clipped by the RuvC1 domain on cas9, and the complementary strand will be clipped by the HNH domain in cas9, forming a double-stranded DNA break (DSB). When cells recognize this DSB, they turn on two pathways for recovering the DSB: non-homologous end-joining (NHEJ) and homology-directed repair (HDR). NHEJ, which is also the knockout system, will produce the error-prone indel mutation, causing the frameshift and the failure of DNA. HDR, which is also called the knock-in system, requires a template strand of DNA. Double-stranded DNA or single-stranded DNA will repair itself by using another strand of DNA as a template, achieving precise gene editing.

Fusing Cas nickase (nCas) or catalytically inactive Cas nucleuses protein (dCas9) with nucleoside delaminate enzyme can produce the base editor and prime editor, inducing single nucleotide site changes without generating DSB in the target sequence or requiring repair templates. There are two types of base editors (BE), cytosine base editors (CBEs) and adenine base editors (ABEs), that can be used. CBEs carry a cytosine deaminase and cause C-to-T on the target sequence. In ABEs, the combination of adenosine deaminase and Cas9 protein induces the conversion of A-to-G transition by catalyzing the oxidative deamination of deoxyadenosine to deoxyinosine [6]. Combining transcriptional repressor or activator domains with deactivated Cas9 (dCas9), which has both cleavage domains, RuvC and HNH domains, inactivated, enables CRISPR-mediated transcriptional activation [7]. Having multiple binding sites in the same promoter can increase CRISPR activation. Multiple transcription factors and cofactors react together to stimulate a higher level of gene transcription. The transcriptional activator that is recruited by dCas9 is commonly VP64. The most effective effector is the CRISPR Synergistic Activation Mediator (SAM) complex, which may recruit MS2, P65, and HSF1 by conbining MS2 with the loop of sgRNA [8]. Such systems are available in the stimulation of single or series gene transcription. The

DOI: 10.54254/2753-8818/21/20230804

binding of a single dCas9 to a promoter of target site can interfere with the binding of transcriptase in space and play a role in inhibiting the transcription of target genes. This process is called CRISPR interference. Fusing dCas9 with the Kruppel-associated box (KRAB) of Kox1, which is a transcriptional repressor or domain, allows the most effective repression of genes [9].

3. Models: disease model and therapy

3.1. In vitro

CRISPR can be applied to cell lines and organoids outside the body. Since the establishment of the first cell line (the HeLa cell line), cell lines have been used more to participate in research [10]. The use of in vitro model systems of cancer cell lines facilitates the study of biological processes and improves outcomes and therapy. Using CRISPR knockin and knockout, we can study the function of genes that affect cancer cells. Using CRISPRa and CRISPRi, you can change the expression level of related genes in cancer cell lines. Compared to animal models, the use of cancer cell lines is less costly and easier to maintain and store. The result is high productivity, and cancer cell lines are amenable to CRISPR.

One important application is to use the CRISPR-Cas9 system to study the relationship between genetic mutations and drug resistance in cancer cells. Drug resistance in cancer cells reduces the effectiveness of drugs and becomes an obstacle to cancer treatment. For example, a knockout in one study using the CRISPR-Cas9 system in the ABCB1 gene (also known as the MDR1 gene) in cancer cells could increase the sensitivity of chemotherapy as well as the concentration of specific chemotherapy drugs in cancer cells [11]. Another study used the CRISPR system with the lung cancer cell line to show a relationship between the NRF2 gene in lung cancer cells, whose upregulation leads to increased drug resistance, and chemotherapy drugs, including Cisplatin [12]. When lung cancer cells are treated with chemotherapy drugs, the volume and proliferation rate of cancer cells are reduced by CRISPR knockout, which means that cancer cells become more sensitive to chemotherapy drugs. CRISPR-Cas9 can create mutations in cancer cell lines in order to study drugs and resistance to that cancer. In one study, the CRISPR system was applied to introduce epidermal growth factor receptor (EGFR) T790M mutations into the PC9 human lung cancer cell line [13]. It was found that the mutation of T790M in EGFR T790M PC9 cells produced by CRISPR-Cas9 is higher than in PC9 cells produced by gefitinib with the same mutation (PC9-g) after long-term exposure. CRISPR could also be used to screen and probe oncogenes for specific cancers [14].

Organoids were gradually built, transforming the 2D medium into the 3D medium. Organoids, which are organ-specific three-dimensional cell clusters, bridge the gap between in vitro model system cell lines or primary cells and in vivo studies because the organoids built up rom adult stem cells are very similar to the tissues of their origin [15]. Because of the heterogeneity and histological features of the primary tumor, PCDOs are an ideal model for the study of anticancer drugs. Organoids can partially simulate the process of cancer and its treatment. Because mouse models differ significantly from humans, the use of patient-derived cancer organoids (PCDOs) would be more precise, and the results of the study could be applied to human bodies. Due to the heterogeneity and histological characteristics of the primary tumor, PCDOs is a suitable model for the study of anticancer drugs [16]. Multiple cancer organoids have been grown for further study of each cancer. It has been demonstrated that induction of NHEJ using CRISPR-Cas9 technology can introduce the required mutations to transform normal organoids and induce tumorigenic growth in xenografts to simulate tumorigenesis [17]. The study targeted genes such as KRAS, APC and p53. Mutations in the KRAS gene, which is an oncogene, are commonly found in colon cancer. This specific genomic alteration is introduced into the KRAS gene of the organoid through HDR-directed DNA repair to establish an organoid model of colon cancer occurrence. Also, researchers can expand and grow primary cancer organoids from cancer patient tissue. The result is an organoid model of cancer that could help researchers use patient tissue directly for targeted studies and rapid and cost-effective personalized drug testing. For example, one study determined the mechanism of oxaliplatin resistance in peritoneal metastases (PM) in colorectal cancer (CRC) and studied strategies to overcome this resistance [18]. They demonstrated that targeting drugs

DOI: 10.54254/2753-8818/21/20230804

using oxaliplatin in combination with redox-targeting drugs have a higher anticancer effect in patients with colorectal cancer by collecting ten stable sources of PM from six patients to establish organoid techniques for studying resistance. CRISPR-Cas9 is often used in cancer organoids to induce NHEJ and HDR mechanisms to disable targeted genes, such as oncogenes, genes that contribute to cancer growth and spread, in order to screen for genes affecting cancer or to develop treatments for cancer.

3.2. Delivery system in vitro

In vitro cell lines, the CRISPR system is typically delivered via microinjection, lentiviral vectors, or electroporation. Mutations in tumor suppressor genes, such as TP53 [19], and uncontrolled expression of oncogenes, such as KRAS [20], in cell lines lead to cancer. Viral vectors, including lentivirus (LV), adenovirus (AdV), and adeno-associated virus (AAV), are commonly used for transport in the CRISPR-Cas9 system in organoids. Viral vectors have significant advantages over other vectors in terms of transport efficiency and tissue specificity. In recent years, because of its unique advantages, AAV vector has been widely used in the development of gene therapy products, and has great potential [21].

3.3. In vivo

CRISPR-Cas9 can also target tumors directly in vivo, which is harder and more challenging. Mouse models are usually used. CRISPR screens for the gene to relate to a specific cancer or test the feasibility of the drug by using CRISPR-Cas9 are two common uses for the application in vivo. In a study, Meiou Dai et al. used the CRISPR system to reveal the vulnerabilities of breast cancer and establish an innovative therapeutic approach through the pharmacological inhibition of torin1-mediated mTORC1/2 and the oncoprotein YAP [22].

Tumor cells are first injected into animal models, usually mice with immune deficiencies. And allow the tumor to develop over time to create an animal model of cancer that can be observed. Injecting the CRISPR system into its animal model, for example by using a viral vector. By knocking out cancer-causing genes, the genes lose their function and thus hinder the development of cancer. Finally, the tumor volume can be measured to determine the feasibility of a treatment or it can be screened for genes that affect the cancer of interest by using simultaneous multi-site targeting genes. A mislink between pieces of DNA from two different genes can lead to a fusion gene, which is an accident that occurs during cell division. They can turn fusion genes and the proteins they encode into factors that trigger tumor formation and are called fusion oncogenes. Fusion oncogenes (FOs) occur in many cancer types and contribute significantly to the development of cancer. Since the elimination of FOs induces apoptosis of cancer cells, a study in 2020 proved that the elimination of FOs can help the treatment of cancer by studying the efficacy of intron-based targeting of transcription factors or tyrosine kinase FOs in reducing tumor burden and mortality in in vivo models [23]. adeno cas9 was injected into Ewing's sarcoma and chronic myeloid leukemia cell lines and mouse models using adv virus vector injection, and was cleaved by targeting FOs introns at both ends. As the cell attempts to repair the break, it joins the cut end, causing the fusion gene in the middle to be completely wiped out.

3.4. Delivery system in vivo

Different target cells will have different suitable delivery vectors. For example, when the target cells are liver cells, plasmids with Cas9 protein genes can be delivered intravenously to the liver, which should use viral vectors or other ways for higher efficiency [24]. The common delivery methods are lipid nanoparticles (LNPs) and viral vectors. LNPs, which are nanoparticles composed of lipid and typically spherical, are a material used for mRNA delivery [25]. Since mRNA has shown great therapeutic potential in many clinical trials and clinical applications, the research and improvement of LNPs will advance the treatment of more diseases. LNPs were applied to mRNA-1273, the coronavirus disease (COVID-19) vaccine approved in 2019 that uses RNA vaccine technology, as used for SARS-CoV-2 which is the virus that causes COVID-19, delivery vehicles, delivering the antibody mRNA [26]. A study conducted in 2020 used CRISPR-Cas9 technology with LNPs and AAV vectors to disrupt the ability of PLK1 gene expression to treat and study cancer [27].

DOI: 10.54254/2753-8818/21/20230804

Viral vector is also a very important transmission medium. The most commonly used Crispr-Cas9 system delivers viral vectors, which are involved in the treatment of many diseases. There are adeno-associated viruses (AAVs), adenoviral vectors (AdVs), and lentiviral vectors (LVs). AAV is a small, uncoated single-stranded DNA virus that has a relatively low probability of immunogenicity, cytotoxicity, and chromosome integration, making it a typical delivery system in vivo [28]. For example, AAV was also used as a delivery vector in a study that reduced PLK1 gene expression capacity, as mentioned above in the last paragraph [27]. AdV as a double-stranded DNA virus can transduce a large number of dividing and non-dividing cells and generate episomal DNA around host DNA without integration into the genome [28]. Thus, ADV can reduce the off-target effect in genome editing. Lentiviral vectors (LVs), which are single-stranded RNA viruses, are gene therapy vectors developed based on the HIV-1, which is the human immunodeficiency virus I. LVs integrate foreign DNA into the host genome, resulting in persistent gene expression.

4. Conclusion

CRISPR-Cas9 is a relatively new gene-editing technique. Different tools added to the Crispr system can generate different functions. As CRISPR-Cas9 expands and advances, it is increasingly making significant contributions to research and treatment strategies for diseases that are inherited or directly linked to genes. It has great potential to be realized in clinical medicine. In cancer, CRISPR-Cas9 has led to a better understanding of what causes cancer and a better way for people to develop cancer drugs. However, CRISPR-Cas9 is a broad and promising technology that will continue to advance and develop more work and research remain to be done to achieve the universal use of CRISPR-Cas9 in clinical treatment. For example, CRISPR-Cas9 has been facing obstacles due to an off-target effect. It will also continue to be explored and studied in the application of cancer therapy.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230805

Current status of drug treatment for hand foot mouth disease

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Abstract. Hand, foot, and mouth disease (HFMD) is an infectious disease caused by the intestinal RNA virus. Hand, foot, and mouth disease can occur every season, with no significant difference in specificity. However, the more frequent season of onset is spring and summer, with April to September as the main month of onset. Coastal areas are relatively popular areas of incidence. HFMD is mainly characterized by herpes with different sizes and clinical manifestations, such as in the hands, feet, and mouth. This article categorizes drugs into two categories: antiviral drugs and antiviral prevention. In describing antiviral drugs, they are classified them into traditional Chinese medicine and non-traditional Chinese medicine. At the same time, the two most effective and commonly used drugs or methods are summarized for preventing HFMD, namely the EV-71 virus vaccine for HFMD, and the washable hand sanitizer that prevents HFMD by blocking the transmission path of the virus. A comprehensive description and summary of the current treatment methods and drugs for combating and treating HFMD. This article provides a comprehensive description of different drugs by elaborating on their advantages and therapeutic principles, combined with their related pharmacological effects.

Keywords: HFMD, Ribavirin, Interferon Alpha 2b Spray, Xiyanping, Vaccine.

1. Introduction

HFMD is a mild infectious viral infection common among young children. HFMD is highly infectious, fast in transmission and wide in approach, and can cause a wide range of epidemics in a short time. The incidence rate will show an explosive growth trend, which has become a major difficulty and hot spot of widespread concern in the whole society [1]. The symptoms are mainly persistent fever, accompanied by sporadic herpes in the mouth, hands and feet, which is relatively high in children under the age of 5. The main route of transmission of HFMD is respiration tract infections. The general preventive measures are to strengthen the establishment of hygiene awareness, such as washing hands before meals. Statistical data show that very few children with severe hand, foot, and mouth disease progress rapidly, leading to critical complications such as meningoencephalitis, myocarditis, and neurogenic pulmonary oedema, endangering the lives of children. The actual infection rate of enterovirus in children aged 5 years and under was 27.74% on average, and the incidence rate of HFMD was 5.80% on average. HFMD has also caused certain economic impacts, and once infected, families of HFMD patients will face certain economic expenses. In 2018, the average cost of non-hospitalized cases was 156.92 ±

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DOI: 10.54254/2753-8818/21/20230805

175.80 yuan/case: among hospitalized cases, the average cost of mild cases was 2247.97=2390.27 yuan/case, and the average cost of severe cases was 14449.45 ± 9826.90 yuan/case, The average cost of death cases is 147-17 million per case. The annual economic loss caused by HFMD is 53-120 million [2].

The main pathogens of HFMD are Coxsackie virus A and enterovirus 71 (EV-A71). Although the majority of HFMD cases are mild and have certain limitations, it is reported that EV-A71-related HFMD has neurological complications [3]. However, monovalent vaccines targeting the EV-A71 virus currently only exist in the Chinese market [3, 4]. EV-71 virus is a non-envelope-positive single-stranded RNA (SSRNA) virus. 5% of its genome ′- UTR is covered by VPg proteins and contains an internal ribosome entry site (IRES) and several clover structures that regulate viral RNA replication and translation [5, 6]. The coxsackie virus that causes hand, foot, and mouth disease includes types 16, 4, 5, 9, and 10 of the coxsackie virus group A, of which the most common is type 16.

Currently, the treatment of HFMD disease mainly focuses on symptomatic and supportive treatment. Currently, there are few specific antiviral drugs, and most of the treatment is through the use of broad-spectrum antiviral drugs., Early use of interferon as a broad-spectrum antiviral drug has significant therapeutic effects [7]. However, interferon is specific in combating enteroviruses and has a high relapse rate after drug withdrawal [8]. The efficacy of interferon alone is not satisfactory, and it has no direct effect on symptoms such as high fever, inflammation, and oral ulcers in HFMD disease. Currently, there are much traditional Chinese medicine and traditional Chinese medicine preparations combined with interferon treatment methods, with significant therapeutic effects. For example, Kangfuxin liquid combined with interferon treatment is both a causal and symptomatic treatment [9]. Then, here are some examples of drugs that protect against the HFMD virus.

2. Antiviral drugs

2.1. Non traditional Chinese medicine

2.1.1. Ribavirin. Ribavirin spray can be used to treat blisters on the fourth day of HFMD, a viral infectious disease caused by RNA virus infection. For antivirals, one option is ribavirin, also known as riboside tourism, which is a broad-spectrum antiviral. However, it is important to note that ribavirin has a number of side effects, especially when administered intravenously, which can produce reproductive toxicity and hematological toxicity and should be considered when using ribavirin in children [10].

Ribavirin is a commonly used antiviral drug that acts as an antiviral by inhibiting inosine monophosphate dehydrogenase, preventing guanosine resynthesis, inhibiting viral replication, indirectly controlling cellular immunity, and protecting virus-infected cells. Ribavirin has shown antiviral activity against a variety of respiratory viruses and enteroviruses. Using poliovirus as a model, it was found that ribavirin can act on the genome of RNA viruses and induce lethal mutagenesis. Poliovirus enters the body's central nervous system through oral transmission, impairs motor function in the spinal cord and causes muscle function to decrease to the point of loss, killing the body. Ribavirin primarily inhibits the RNA-dependent RNA polymerase of poliovirus so that poliovirus can induce mutations to generate new RNA templates and act as an antiviral. Coxsackie virus (CV) belongs to RNA viruses, such as CVB3 can induce direct cell damage, and then cause target organ tissue damage and dysfunction through the interaction of human inflammatory response to CVB3 infection and cell death. Ribavirin inhibits viral uptake by binding to the capsid of the virus, thereby inhibiting viral replication and reducing its transmission. CVA10 can also cause HFMD. Ribavirin was found to be effective in the treatment of HFMD, significantly inhibiting CVA10 with an in vitro inhibition rate of 32% and in vivo protection rate of 60%, indicating that ribavirin is able to inhibit CVB replication [11].

Ribavirin is rapidly absorbed after oral administration (maximum concentration time =1.5 hours), followed by rapid distribution and extended elimination phases. Absorption in the proximal small intestine is active via a concentrated N1 sodium-dependent nucleoside transporter. Ribavirin appears to be widely absorbed. However, absolute bioavailability is around 50%, presumably due to primary

DOI: 10.54254/2753-8818/21/20230805

metabolism. Because ribavirin is distributed via ES- nucleoside transporters to non-plasma (cellular) compartments, its surface distribution volume is wide (about 2000 L). The ribavirin does not bind to plasma proteins. After multiple doses, there is a large build-up in the plasma that takes about four weeks to stabilize. The multi-dose half-life is approximately 298 hours due to the slow elimination of ribavirin from the non-plasma compartment. The pharmacokinetic properties of ribavirin in particular populations, the effects of food on the pharmacokinetics of ribavirin, and potential interactions between ribavirin and other preparations are also reviewed.

2.1.2. Interferon alpha 2b spray. Interferon α is an important immunoprotective cytokine that can only effectively bind to cell surface receptors, express antiviral proteins, inhibit virus replication, and prevent the virus from invading normal cells. It can also enhance cellular immunity and promote the proliferation of cytotoxic T lymphocytes, with significant viral clearance.

Recombinant human interferon is used in children with HFMD α- The clinical efficacy of 2b aerosol inhalation treatment is significant [12]. Administration of recombinant human interferon to children with HFMD α- After 2b aerosol inhalation therapy, the drug can play a protective role in the process of enterovirus infection, directly clearing the invading RNA virus, and does not require the joint involvement of the immune system, resulting in no drug resistance. The spray inhalation of interferon also avoids various symptoms such as chills, muscle soreness, and fever caused by intramuscular or subcutaneous injection [12].

2.2. Traditional Chinese medicine

2.2.1. Xiyanping. Xiyanping is produced by using modern technology to extract andrographolide from the entire leaves of the plant Andrographis, and through a unique sulfonation patented process.

A water-soluble traditional Chinese medicine injection, which changes the structure of andrographolide components themselves and enhances their pharmacological effects due to structural changes [13]. The drug concentration in the blood is higher, and it can well penetrate viral cells, occupy the binding sites of viral replication DNA and protein, and prevent protein from wrapping DNA fragments, making the virus unable to replicate, thereby inhibiting or killing the virus. Xiyanping has pharmacological effects such as antiviral, enhancing immunity, clearing heat, and anti-inflammatory, which is beneficial for disease recovery. The study used a control method to compare the effective rates of Xiyanping and ribavirin in the treatment of patients with non-severe HFMD. The effective rate of Xiyanping was 94.28%, while the effective rate of ribavirin was 70% [14]. And this result has statistical significance after statistical verification. In addition, in the study, patients treated with Xiyanping had shorter fever relief time, rash resolution time, stomatitis healing time, and hospital stay than those in the control group treated with ribavirin [14].

2.2.2. Treatment of HFMD with combination of traditional Chinese medicine. For the treatment of HFMD, conservative treatment is mostly used in medicine. However, compared with Western medicine, which has many adverse reactions to the human body, Chinese medicine is very popular among patients for its mild medicinal properties and low medicinal cost. In 2020, 70 children with HFMD of damp heat and steaming type were admitted to Luxi County Maternal and Child Health Hospital in Jiangxi Province, China, which proved the value of Qingwen Baidu decoction combined with traditional Chinese medicine fumigation for HFMD of damp heat steaming type. All patients and their family members excluded special cases. The control group chose the treatment method of traditional Chinese medicine fumigation and washing, and observed that Qingwenbaidu Decoction combined with traditional Chinese medicine fumigation and washing was used for treatment. consistent. After careful comparison of the time of the disease's disappearance, immune function and other conditions, the researchers concluded that clinical TCM treatment of HFMD should choose the method of dispelling dampness, clearing qi, clearing away heat and detoxification; Qingwenbaidu drink with fire function. They can be used in combination with western medicine to alleviate the damage to the human body

DOI: 10.54254/2753-8818/21/20230805

caused by the excessive therapeutic effect of western medicine [15]. Similarly, from 2018 to 2019, Huangpu People's Hospital of Zhongshan City, Guangdong Province, China adopted 78 patients with HFMD, treated with western medicine ribavirin as a control, and compared Yinqiao Haoqin Decoction combined with traditional Chinese medicine acupoint application in the observation group. Keeping other observations on the treatment effects of the two groups and the safety of the medication, the researchers concluded through comparison that the latter traditional Chinese medicine therapy makes the medication safer and the time for the disease to subside will be significantly shortened (for example, the patient's fever will go down faster, Herpes dissipates faster), has more definite curative effect and faster recovery of patients [16]. Xiyanping injection was also widely used in the medical field for HFMD. The fumigation treatment in traditional Chinese medicine can also fit the treatment concept of viral infection. Yingtan Hospital of Traditional Chinese Medicine, Jiangxi Province, China, from September 2014 to 2015 In February 2010, 40 children with HFMD were randomly selected. The experimental group and the control group had the same number of people and the age range was 1.8 to 2.9 years old. The experiment was carried out after the researchers confirmed that there was no statistically significant error. All variables such as the basic treatment course and drug dosage of the control group and the experimental group are the same, and the experimental group uses the medicine fumigation and washing made of various medicinal materials once a day. The result is that all the data of the experimental group are superior to those of the control group. It is concluded that the treatment of traditional Chinese medicine has been developed since ancient times and has gradually matured. Many experiments and real data have proved that the combination of traditional Chinese medicine and Western medicine is handicap. Mouth virus and even more other types of viruses have good curative effects, which also suggests that medical workers can more vigorously promote the rational use of traditional Chinese medicine in the course of treatment [17].

3. Medications for preventing hand, foot, and mouth disease

3.1. TJAB1099

EV-71 virus is mainly transmitted orally through direct contact with oropharyngeal secretions and faeces through contaminated objects. Therefore, blocking this route of transmission is an effective way to prevent the spread of the virus. Although patients and non-patients usually have certain isolation measures. Routine methods such as isolation, ventilation, and surface disinfection are used to control the spread of the virus. However, the disinfection effect of these methods is very limited, and EV71 is mainly transmitted through direct contact type direct transmission. Vulnerable populations, such as children, have the habit of not washing their hands and sucking their fingers, which is the most important reason for the spread of the virus among children. Therefore, hand disinfection is the most direct and effective method to block the spread of EV71. EV71 is a non-envelope virus. Eliminating a series of EV-71 viruses targeting the hands can effectively inhibit the spread of the HFMD virus.

However, due to the high stability of EV71, the inactivation effect of traditional disinfectants such as alcohol on EV71 is not ideal. A new pyridyl imidazol idone compound (TJAB1099) can specifically inhibit the replication of EV-71 in vitro. However, TJAB1099 is insoluble in water, resulting in extremely low bioavailability, and is not suitable for oral absorption [18].

A washing-free gel containing TJAB1099 was developed because Carbopol semi-solid gel is easy to dissolve with TJAB1099 in ethanol, and it will quickly liquefy after being heated by hand contact. This makes the development of TJAB1009 wash-free gel feasible [18].

3.2. vVaccine

The research and development of the EV71 vaccine mainly include inactivated whole virus vaccine, attenuated live vaccine, virus-like particle vaccine, and DNA vaccine. Currently, three companies in China, including the Institute of Medical Biology of the Chinese Academy of Sciences, Beijing Kexing Biotechnology Co., Ltd., and China Biotechnology Group Wuhan Institute of Biological Products, have approved the listing of EV71 vaccines.

DOI: 10.54254/2753-8818/21/20230805

The meta-analysis results of the EV71 vaccine have been verified in the EV71 vaccine-vaccinated population [19]. The whole genome microarray was used to detect the changes in the transcriptome of peripheral blood mononuclear cell during the initial immune response and re-response of the new EV71 vaccine. It was found that the initial immune response could activate the type I interferon and antiviral response pathway, while a stronger type I interferon and antiviral response, inflammatory response, and humoral immune response was only observed during the re response [19]. In some studies, all healthy children aged 6 to 59 months who were vaccinated with the EV-A71 inactivated vaccine in the vaccination clinics in 89 counties (cities, districts) of Zhejiang Province from April 2016 to March 2018 were selected as the study subjects. The local and systemic adverse reactions of the vaccinators were collected through observation within 30 minutes of the vaccination site, follow-up within 3 days, and 4 to 30 days of vaccination, respectively. A total of 71663 doses of EV-A71 vaccine were administered, with no significant difference between the doses administered to boys and girls; The 6 to 11, 12 to 23, and 24- to 59-month-old groups were vaccinated with 13707, 32639, and 25317 doses, respectively. The incidence of adverse reactions was 0.33% (239 dose times), 1.58% (1133 dose times), and 0.34% (244 dose times) within 30 minutes, 3 days, and 4 to 30 days after inoculation, respectively. A total of 1372 dose times of adverse reactions occurred within 3 days, with an incidence of 1.91%. Among them, 539 dose times of first-level adverse reactions occurred, 677 dose times of second-level adverse reactions occurred, and 156 dose times of third-level adverse reactions occurred. There were no fourth-level adverse reactions. Among local adverse reactions, redness, induration, and pruritus are more common, with an incidence of 0.05% (39 dose times), 0.02% (16 dose times), and 0.02% (12 dose times), respectively. Among systemic adverse reactions, fever is the most common, with an incidence of 1.19% (856 dose times), followed by diarrhoea and decreased appetite, with an incidence of 0.15% (104 dose times), and 0.13% (90 dose times), respectively [20]. The adverse reactions after vaccination with the EV-A71 vaccine are mostly mild and common, with no rare adverse reactions found.

4. Conclusion

Drugs for HFMD is actually a lot. Currently, there are no specific drugs to treat HFMD-related viruses. Generally, prevention of HFMD is implemented through both vaccination and attention to personal hygiene to block the transmission path. However, at this stage, only the EV71 vaccine is approved by the CFDA (China Food and Drug Administration). There are more than 20 enteroviruses that can induce HFMD, so the EV71 vaccine cannot completely prevent the occurrence of hand, foot, and mouth disease. For antiviral drugs that inhibit viral replication or destroy viral proteins, broad-spectrum antiviral drugs, such as ribavirin, are commonly used in clinical practice. Ribavirin can develop resistance, so it is necessary to control the dosage of each intake. The main drugs for HFMD that improve the immune system and strengthen resistance are interferon. In China, doctors will use traditional Chinese medicine or traditional Chinese medicine combined with antiviral drugs to treat HFMD, which has a significant role in antiviral and immune enhancement. With the invasion of hand, foot, and mouth disease virus into the human body and its auxiliary mechanisms gradually being thoroughly studied, inhibiting the replication of HFMD virus has become an important idea in the research of HFMD-specific drugs. It is believed that more specific drugs and vaccines related to HFMD will be developed in the future, and people need to maintain our expectations.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230807

The role of the 14-3-3 protein family in disease

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Abstract. 14-3-3 protein is a dimer protein extracted from bovine brain cells, a highly conserved protein prevalent in eukaryotes. There are seven subtypes in mammals, respectively σ, ζ, β, γ, η, ε and τ. The subtypes differ in content and function. It was found that the protein interacts with corresponding ligand protein in regulating life activities, such as: cell cycle regulation, cell development, the transmission of cell signal molecules plays an important role. At the same time, related regulators have been developed based on cell-cell interaction. In recent years, the exploration of 14-3-3 protein has been deepening gradually. Inhibition or stabilizers of the 14-3-3 protein in question have been studied or discovered, and have become a new target for treating diseases. This paper reviews the effects of 14-3-3 protein on neurological disease, cardiovascular and cerebrovascular diseases and cancer are reviewed. It is found that protein subtypes affect the occurrence of diseases.

Keywords: 14-3-3 protein, disease, neurological disease, cardiovascular disease and cerebrovascular disease, tumors.

1. Introduction

In 1967, Moore and Perez first discovered the 14-3-3 protein in cow brain monomers, at the time its designation depended on the number of DEAE-cellulose fragments and the location of migration in gel electrophoresis, and the molecular weight of the monomer is 27-31 KDa. It is ubiquitous in eukaryotic cells. It is also a kind of a highly conserved and acidic heterodimer protein. Seven protein subtypes have been found so far in mammals, respectively are σ, ζ, β, γ, η, ε and τ. The differences between different isoforms are mainly reflected in facultative grooves of protein structure, there are binding sites to different ligands in facultative grooves-it is important structure for the14-3-3protein. Studies have shown that the 14-3-3 protein can regulate the function of different proteins through cytoplasmic isolation, regulation of enzyme activity, prevention of degradation, and promotion of protein modification, so the 14-3-3 protein and the corresponding ligand protein play an important role in different areas of life, such as: regulation of metabolism, cell cycle regulation, cytoplasmic transport transcription, malignant tumor suppression and so on [1,2]. Meanwhile, in the area of protein-protein interactions (PPIs), the 14-3-3protein family was also the midpoint of the study. By combining small molecules or proteins with 14-3-3proteins, new drug nave been developed to tread various diseases. There are hundreds of small molecules that can bind to 14-3-3proteins, known inhibitors and stabilizers are 14-3-3 protein and chaperone protein PPI types, R18 is the first protein inhibitor to be discovered that binds to this protein and has inhibitory effect on tumor cells. Subsequently, lacoceramide, HSP20,

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DOI: 10.54254/2753-8818/21/20230807

FOBISNI101 and other small molecules were identified by scientific and technological means to bind to 14-3-3 protein. Clostridium A, CotyledoninA and semi-synthetic clostridium extracted from natural products can be used as the stabilizer of14-3-3protein [2]. In recent years, 14-3-3 protein has become one of the important proteins in the study of novel objects. This paper reviews the effects of protein families on different diseases.

2. Diseases of nervous system

14-3-3 protein is widely distributed, the most abundant is found in nerve tissue cells. It plays an important role in the expression and function of nerve cells [3]. Common neurological disorders include Parkinson's disease (PD), Alzheimer's disease (AD), Depressive disease (MDD) [3-5].

2.1. 14-3-3 protein and Parkinson's diseases

When dopaminergic neurons disappear in the cerebral cortex and substantia nigra and ubiquitin-positive protein inclusion functions appear in the remaining dopaminergic neurons (Lewy corpuscles), people will suffer from PD. The main components of the Lewy corpuscles are σ-eukaryotic protein. Some studies have found that Lewy corpuscles contain 14-3-3 ε, τ, ζ, η subtype protein. On the one hand, these subtypes can combine with σ-eukaryotic protein to form structures similar to Lewy corpuscles, so it reduces the Lewy corpuscles. On the other hand the ζ subtype protein binds to the dopamine rate-limiting enzyme Tyrosine-Hydrogenase (for short TH) to promote dopamine synthesis. Because the σ-eukaryotic protein and 14-3-3 protein sequences have a certain similarity, so it also binds to TH, which effects dopamine synthesis. In the interaction of the three on dopamine, that it plays an important role in the regulation of PD patients [3].

2.2. 14-3-3 protein of Alzheimer's disease

AD disease is associated with the formation of neurofibrillary tangles (for short NFTs) from the aggregation of Tau proteins associated with paired spirals of hyperphosphorylated microtubules. NFTs contain five different subtypes of protein. Tau proteins are stimulated by protein kinases activated by 14-3-3ζ proteins, so it destabilizes the Tau proteins and cause structural variation, leading to NFTs, and increasing the incidence of AD disease [3].

2.3. 14-3-3 protein and depression

Some studies have found that hippocampal glucocorticoids increase after stress, which is easy to produce MDD disease. Ginsenosides showed protective effect on hippocampal nerve cells after stress. 14-3-3 protein has a strong affinity with panoxadiol, the main ingredient in ginsenosides [5]. It reflects that the 14-3-3 protein may be to prevent or treat depression.

3. Cardiovascular and cerebrovascular diseases

The occurrence of cardiovascular and cerebrovascular diseases is caused by atherosclerotic blood vessels. If it occurs in the heart, it is cardiovascular disease, while if it occurs in the brain, it is cerebrovascular disease.

3.1. 14-3-3 protein and diabetic cardiomyopathy

The scientists compared mice genetically modified for DN14 3 3ηsubnovel protein with wild mice that developed diabetes. No significant difference in blood sugar was observed but the loss of heart function was significantly greater in mice that lacked14-3-3 protein. The immunofluorescence of AsK1 is obvious in the left ventricle, the myocardial cells are enlarged, and the content of other substances is different. One of the reasons why macrophages become polarized is that fatty acid metabolism is altered during DCM. Meanwhile, the anti-inflammatory M2 type is weaker than the anti-inflammatory M1. Both metabolic polarization and macrophage polarization during DCM can be mediated by14-3-3 protein. It has been found that a high-fat diet in mice lacking14-3-3 protein increase the expression of the M1-macrophage marker protein, and the expression of anti-inflammatory proteins was reduced. So people get inflammation. The lack of the 14-3-3 subtype protein may be regulated through other signaling pathways in the body. It causes stress in the endoplasmic reticulum. which promoting myocardial

DOI: 10.54254/2753-8818/21/20230807

apoptosis in diabetic mice. The difference of 14-3-3 protein expression based on the study data can provide a new idea for the study of blood glucose diseases. It is also possible to further control the prevention and treatment of diabetes by regulating the AsK1signal through 14-3-3 protein, or regulating the expression of M1 and M2 -type cell marker protein in macrophages [6, 7].

3.2. 14-3-3 protein and atherosclerosis

Atherosclerosis is the swelling of the endothelium of the arteries, leading to narrowing and hardening of the blood vessels. The adhesion of platelets to other secretions is one of the causes of disease. Special granule and compact granule are two types of platelet granule, and 14-3-3ζ protein subtype is mainly distributed in dense particles, and have the function of activating platelet secretory protein to promote atherosclerosis. And research have found that the presence of protein is found in the atherosclerotic plaque of the human abdominal aorta [7, 8].

4. Cancer

The imbalance between cell death and cell proliferation is one of the important causes of cancer [9]. At present, 14-3-3protein subtype has been found to be expressed in liver cancer, rectal cancer, lung cancer and other cancer cells. Different subtypes have different effects on different cancers [8].

14-3-3η protein and prostate cancer

The factors that determine the formation, metastasis and mutation of prostate cancer cells are androgens. The androgen-dependent character of prostate cancer was discovered by Huggins and Hodges in 1941. Androgens bind to estrogen receptors (AR) and act similarly to transcription factors, so they can regulate the expression of a large number of genes. Removal of testicles is a clinical treatment for prostate cancer. But there is a possibility of relapse. Subsequent studies by Mark et al. on recurrent prostate cancer cell line CMR-R1 found that endogenous AR receptors were activated by protein 14-3-3η at low oxygen dihydrotestosterone concentrations, and at the same time, enhance the role of AR. Androgen increase the expression of 14-3-3η, and 14-3-3η played a regulating role in AR. Several months were observed in mouse castration experiments, 14-3-3η protein content changed but eventually returned to pre-castration. It is known that androgen changes or deficiencies occur in prostate cancer that the 14-3-3η protein acts as an androgen agonist [8]. This could lead to new ways of treating prostate cancer.

14-3-3Σ protein and cancer

After DNA damage, 14 3 3σ protein is the only subtype that can normally inhibit cell growth and cell cycle progression. P53 is a tumor suppressor that can be activated after oncogene damage or disorder which stops the cell cycle in its tracks and programmed cell death, and at the same time inhibit the formation of other tumor cells [2]. It is a key factor affecting tumors. P53 can induce the expression of 14-3-3σprotein in DNA damage [10]. Upon induction 14-3-3σ binds to and sequesters the cyclin G2 complex in the cytoplasm, and prevents the nuclear localization of mitosis, so that the cell cycle to stall. This allows damaged DNA to be repaired before the cell cycle progresses. The results indicate that 14-3-3σprotein inhibited tumor activity [2,10,11]. In addition, 14-3-3σ protein is down-regulated in dysplastic tissues and cells, and it can be seen that early signs of tumor development may be reduced function of 14-3-3σ protein [12].

5. Conclusion

In recent years, the research on14-3-3protein has been deepening gradually, and the mechanism of action of the protein on the disease has been discovered. 14-3-3 protein originally found in the brain of cattle, so it is most closely related to the brain. It plays an important role in the function of the nervous system, such as: nerve signal conduction, nerve cell development, nerve cell connection and so on. In addition, further research has also shown that the 14-3-3protein family has important function in different parts of the body. They interact with other proteins. The regulation of cell cycle, cell apoptosis and signaling molecules are all dependent on14-3-3protein. At the same time, 14-3-3 protein is a new target for developing drugs to treat disease. Not only can affect the nervous system, cardiovascular

DOI: 10.54254/2753-8818/21/20230807

cerebrovascular, tumor cells, but also has unknown effects on other diseases. Although some of the functions of14-3-3protein have been discovered and applied, the regulation of 14-3-3protein on human function is not fully understood. For example, no key drug has been developed to treat a disease with protein as the main body, and the mechanism of action of protein subtypes on different subtypes of the same disease. The 14-3-3protein is a large family, and as technology continues to improve, its role in diseases and improve life activities will eventually be discovered.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230809

An introduction to cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade

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Abstract. Cancer immunotherapy has been a hot topic of cancer therapy discussion for over decades. Several successful cancer immunotherapies have already existed for about 30 years, however it is just in the past decade that immunotherapy has achieved broad breakthrough on patient survival in multiple high-incidence cancer indications. Immunotherapy, as a promising therapy depending mainly on the mechanism that immune cells work to eliminate cancer cells, has three hot topics recently. Cancer vaccine is a therapeutic vaccine that typically involves exogenous administration of selected tumour antigens to activate dendritic cells (DCs), or even DCs themselves in order to initiate and stimulate immune response to tumour cells, regain their control over tumour growth, induce existed tumour regression and eradicate minimal residual disease. Chimeric antigen receptor (CAR) T-cell therapy uses a patient's own T cells, but genetically engineered to express a synthetic receptor that binds to a tumour antigen more precisely and efficiently, to serve as more effective army against tumours. Immune checkpoint blockade (ICB) depends on blocking certain receptors and their ligands involved in pathways that attenuate T cell activation — for example, cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1) and its ligand, PDL1 — to restore T cells' activity and prevent acquired peripheral tolerance to tumour antigens. This review gives a brief introduction to how human immune system works and a basic overview on the principles of cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade (ICB).

Keywords: immunotherapy, cancer vaccine, chimeric antigen receptor T-cell, immune checkpoint blockade.

1. Introduction

Our body is facing all kinds of threats from both inside and outside our body. It is the fundamental responsibility of immune system that every part of our body is completely under its surveillance. When certain normal cells mutate into cancer cells, it's our immune system that immediately eliminates them. However, sometimes cancer cells find their way to escape surveillance from immune system. That's how cancer happens.

As mentioned above, the immune system retains the capacity to kill cancer cells itself. The core principle of immunotherapy of cancers, which has made a broad impact on cancer treatment these years, is to restore the activity of immune cells against cancer cells or enhance the pre-existing immune response through external intervention. This review mainly focuses on three hot topics in this

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DOI: 10.54254/2753-8818/21/20230809

field, that is cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade. Cancer vaccine uses cancer-specific antigen as a stimulation to boost immune system to find and destroy tumours. It's only used in person who already has tumours so that it's called therapeutic cancer vaccine. CAR-T cell uses a kind of T cells that more precisely target on cancer cells. This special kind of cells are programmed from normal T cells, researchers load chimeric receptors onto the surface of the T cell to make it more precise and effective. Immune checkpoints are referred to some suppressers in the immune system, which is originally used to attenuate excessive immune response, however exploited by cancer cells to escape immunity. Many researches try to find those molecules called immune checkpoint inhibitors to block this checkpoint and restore immunoactivity, which works upon blocking certain checkpoint-associated proteins from binding with their receptors.

2. A brief introduction to human immune system

Human immune system is an army inside our body, mainly functioning in preventing foreign pathogens from infecting our cells and clearing those infected or aging or other abnormal cells like cancer cells. It mainly consists of all kinds of physical barriers like skin or mucous membranes, immunological molecules like antibodies and lysozymes and immune cells like T cells, B cells, phagocytic cells, dendritic cells, natural killer cells and so on.

When an invasion of pathogen happens or an abnormal cell appears, the immune system immediately detects it respond to it. The response can be divided into innate immune and adaptive immune. Innate immune exists inborn, acting as a protective and clearing agent against all kinds of pathogens without specificity. This is the first defense line and the second defense line of our body. The first line of human immune system consists of skin and mucous membranes, which provide physical barrier and secretions like gastric acid to kill pathogens. The second line consists of Phagocytic cells and lysozyme, "wandering" inside human body, detecting and killing pathogens without specificity. These two lines are called innate immune, because it's born with nearly everyone and is not against to any specific pathogen.

But there are threats that this non-specific innate immune cannot handle. A kind of cells called antigen presenting cells (APCs) in our body can present antigens and initiate adaptive immune against specific pathogen. Higher efficiency and specificity are its features. This is the third line, also the most important line in our body, with participation of all kinds of immune cells.

2.1. Discrimination

Immune system needs to tell the differences between normal cells and cells infected by pathogens or those with 'sickness' like cancer. The way they use differs between innate immune and adaptive immune. The innate immune system recognizes pathogens by their conserved features through a sort of receptors called pattern-recognition receptors (PRRs), the most representative recognition receptors in innate immune, which are highly conserved in evolution. The features that PRRs detect include pathogen-associated molecular patterns (PAMPs), such as bacterial and fungal cell-wall components and viral nucleic acids, or damage-associated molecular patterns (DAMPs), such as heat shock proteins and IL-1α. This nonspecific detection of PAMPs and DAMPs by PRRs leads to the induction of inflammatory responses like pyroptosis and release of IL (interleukin), and further innate immune responses. Especially the sensing of microbes by PRRs expressing on APCs, particularly dendritic cells (DCs), directly leads to the activation of adaptive immune responses [1].

The recognition of pathogens by the adaptive immune system is mainly mediated by APC and major histocompatibility complex (MHC). MHC is a protein family located on animal cell surfaces, always combined with cell antigens. Cells infected by pathogens will present abnormal MHC molecules recognized by immune cells, mostly APCs, so the immune system knows what's wrong with those cells. Then they digest and process pathogens' antigens into short peptides, exposing and presenting them to other immune cells like T cells.

Common APCs include phagocytes, B cells and dendritic cells (DC). etc. Among all kinds of APCs, DC is the most efficient in presenting antigens to other immune cells. An activated DC acts as the core

DOI: 10.54254/2753-8818/21/20230809

of priming and maintaining adaptive immune response through induction of effective and persistent immune cell activity in participation with CD4+ T helper cell and cytotoxic T lymphocyte (CTL) responses, recruitment of T cells and B cells and durability and maintenance of response [2]. After antigen presentation, it's T cell' s and B cell's work to attack pathogens.

2.2. T cell response

T cells mature in thymus where the name comes from. In a healthy person' s body, these T cells act as military that keep moving between lymph nodes and the blood and the APCs were tested by T cells in case of signs for damage or infection [3]. After recognizing antigens presented by APCs depending on the T cell receptors (TCR), T cells initiate division and differentiation into different effector T cells, including cytotoxic T cells, helper T cells and regulatory T cells.

Cytotoxic T cells (also called killer T cells) attack infected cells directly through direct contact and cytokines released to initiate apoptosis. Helper T cells promote maturation and function of other immune cells like B cells differentiation and antibody secretion. Suppresser T cells can inhibit other immune cells' functions, preventing abnormal immune response to normal cells. They are the most important attacker against tumours.

2.3. B cell response

B cells can also act as one kind of APCs. After stimulated by antigens and helper T cells as followed, B cells go through differentiation and become plasma cells and memory B cells. Plasma cells secret a special protein called antibody, which originally starts as a cell-surface receptor [the B-cell receptor (BCR)]. so that it has high specificity to certain antigen and tightly combines with it, inhibiting its activity and assisting T cells to attack pathogens. And also these antibodies are able to function normally in tissues where T cells do not. Once they are produced by B cells, they are able to bind to pathogens that escape from the first two barriers. Another function that is easily ignored of antibodies is to neutralize soluble poisons (toxins) from some attackers, which shows importance in many cases, for example, in the response to diphtheria. After the response the antibodies keep existing in the blood for a period of time, also found within the mucus that lines our gastrointestinal organs and in interstitial tissue fluids, which is related with immune system's memory [3].

2.4. Memory

Memory is another representative feature of immune system. It means individuals who get rid of a specific pathogen at the same time gain the ability to resist reinfection with the same pathogen for a period of time. It just seems that the immune system builds memory about the pathogen after one infection. This marvelous capacity can save resources for our immune system, and it is also the key to vaccination.

Immune system uses many ways to keep memory about once-met antigens. Following an infection (or a vaccination, using antigens to stimulate the foundation of immune system's memory), antibody levels rise at first as immune response to antigens, and then fall off to lower levels that are higher than in the naive state however and keep themselves in that way for a long period of time [3]. This indicates that some antibodies remain to respond to a new infection about to happen in the future.

Besides antibodies, other important differentiations occur in different populations of antigen-specific lymphocytes like T cells and B cells. Part of B cells after stimulated differentiate into memory B cells which do not actively secrete antibodies immediately, but remain in the body long-term retaining the 'potential' to produce antibodies when a new infection occurs. It also has been proved by experiment that T cells which has 'met' the same antigen before are easier to activate, initiating response to the same pathogen.

3. Therapeutic cancer vaccine

Just like its defense function against foreign pathogens, immune system retains the ability to kill tumour cells, so there exist vaccines for cancer. Cancer vaccines according to its treatment aim can be

DOI: 10.54254/2753-8818/21/20230809

divided into preventative cancer vaccines and therapeutic cancer vaccines. Preventative cancer vaccines are vaccines that can protect healthy people against certain cancers, mainly caused by certain kinds of viruses. This type of vaccine only works when the person has gotten the vaccine before they are infected, just like normal "vaccines" you have heard. There are already 2 types of vaccines that prevent cancers have been approved by the U.S. Food and Drug Administration (FDA): HPV vaccine and Hepatitis B vaccine.

Therapeutic cancer vaccines are used for people who already have tumours, working to promote immune system to fight cancer. Different treatment vaccines deal with tumours in different ways. They can also stop a tumour from growing or spreading, keep destroying cancer cells still surviving after treatment and prevent the cancer from coming back. For most types of cancer vaccines, CD8+ cytotoxic T cell-mediated cellular immunity is especially significant in eliminating malignant cells for cure [4].

3.1. Principle

Most of cancer vaccines typically refer to the application of selected tumour antigens just like other vaccines against viruses do. Moreover, it is generally combined with adjuvants that are used for activating DCs, or even DCs themselves, the aim of which is to stimulate DCs' function that recruits, activates and maintains T cell response against specific tumour antigens, finally regaining control over tumour growth. The main basic concerns needed for successful therapeutic vaccination against tumours include delivery of antigens with both large amount and high quality to DCs, optimal DC activation, DC functioning in promoting strong and sustained T cell responses and durability of response and maintenance of effection [4].

3.2. Antigen selection

We can use the term 'neoantigen' to refer to those mutated tumour antigens that only express in tumour tissue. Neoantigens are typically not germ line encoded. Therefore, theoretically, the patient possesses no central tolerance towards these antigens, which means the existence of neoantigens is probable to arouse a robust T cell response [5]. Recently several clinical trials using neoantigens have indicated that patient survival increases, which can be taken as evidences of their immunogenicity. For example, a single-arm study uses monocyte-derived DCs loaded with personalized neoantigen short peptides on patients with melanoma demonstrating that neoantigen vaccines are able to induce a T cell-specific immune response [6]. Besides neoantigen vaccine, shared-antigen vaccine may be a better choice for those not suitable for the former. Some researchers attempt to include neoantigens along with shared antigens to expand the application scope of neoantigen vaccination [4].

3.3. Administration of antigen

So far, effective sources of tumour antigen delivery have included DNA, RNA and synthetic long peptides (SLPs) [4]. DNA vaccines can direct synthesis of antigen peptides, however requiring transcription, translation and process after injection. Certain advantages of DNA vaccines are that they are easy to manufacture, carrying built-in adjuvants and able to synthese large amounts of antigens [7]. RNA vaccines are similar, however without need for transcription and thus it directly goes to antigen protein translation and processing and presentation on MHC molecules. SLP vaccines can be directly recognized by DCs, the greatest advantage of which compared with short peptide vaccines is its requirement for DCs' processing before combining with MHC, thus increasing the treatment's efficiency.

3.4. Tumour resistance against vaccine

The resistance of tumour against therapeutic cancer vaccine can be divided into two mechanisms. One is tumour cell intrinsic mechanisms, mainly including resistance strategies conducted by those tumour cells themselves. Tumour intrinsic mechanism mainly include the downregulation or lack of tumour antigen expression [8], alterations in the antigen processing pathway and loss of HLA expression [9],

DOI: 10.54254/2753-8818/21/20230809

all of which to the end prevent recognition of tumour cells by T cells [4]. Another mechanism is tumour cell extrinsic mechanisms, mostly correlated with the tumour stromal components that downregulate T cell response. Tumour extrinsic mechanism include accumulation of immunosuppressive cells in TME (tumour microenvironment) such as Treg cells, MDSCs, tumour-associated macrophages (TAMs) that immune system has prepared in case of need for downregulation of T cell activation.

Because of the existence of immune escape in tumours, single vaccine hardly works. Use of combined therapy with cancer vaccines and other therapy like ICB or traditional chemotherapy are more common. Adjuvants are also introduced usually, roles of which include maximizing DC activation, promoting recruitment and activation of T cells, keeping activity of immune cells, etc.

4. Chimeric antigen receptor T cell (CAR t-cell)

As followed, immune cells inside our body originally have the ability to kill the tumour, among which T cells act as the main force when attacking tumours. Unfortunately, tumour cells have their methods to escape T cells, which called immune escape, a key mechanism of which is to reduce T cells' affinity for tumour antigens. This ability also influences the effectiveness of immunotherapy applied to the patient, leading to tolerance in the setting of cancer immunotherapy. The adoptive transfer of autologous T cells retrofited by genetic engineering to express more 'powerful' receptors targeting molecules expressed on malignant cells may have greater potential for cancer therapeutics compared to the approaches above [10].

4.1. Principle

As is known to all immune cells have the ability to kill tumour cells, but tumour forms when somehow they lose the power to recognize. To enhance TCR's affinity for a tumour antigen, researchers redirect and reprogram T cells with a special receptor called chimeric antigen receptors (CARs).

Chimeric antigen receptor is a kind of recombinant receptor which provide both antigen-binding and T-cell–activating functions. Over the past decade has reported a multitude of these so-called CARs, targeting an array of tumour antigens on tumour cell surface [11]. Typically, a CAR possesses a single chain variable fragment (scFv) originally from the antibody targeting the tumour antigen with affinities several orders of magnitude higher than normal TCRs, which gives these CAR T-cells the ability to be insensitive to most tumour escape mechanisms, mostly related to MHC loss.

4.2. Generation of CAR T-cells

Researchers typically generate CAR T-cells by the following steps: collecting cells from the patient by leukapheresis, removing myeloid cells by elutriation, enrichment of T cells, genetic engineering and ex vivo cultivation for expansion. The most critical constraint in cell manufacturing is isolation of T cells from leukapheresis samples. Typical use of positive and negative selection methods may inevitably cause T lymphocytes enriched to still be mixed with inhibitory cell types that may impede further CAR T-cell expansion in culture [10]. However, a recent case was reported that during T-cell sample processing an leukemia cell was unexpectedly transduced by the CAR transgene, resulting in these receptors binding to the CD19 epitope on the surface of the leukemia clone that had expanded massively in an ALL patient, making it unrecognizable by anti-CD19 CAR T-cells [12]. Such cases indicate that more efforts should be focused on the purification of T cells to better improve the safety and efficacy of this type of therapy.

4.3. Structure of a chimeric antigen receptor

The structure of CAR is another key factor for enhancing affinity to tumour antigens and the following immune response. The first generation of CAR mainly consists of two parts. The extracellular part is tumour-antigen-combine domain originally from a scFv, and the intracellular part is CD8 and the CD3ζ signaling chain that mediate T cell activation just like how TCR does. The second generation, in order to further strengthen T cell function after antigen recognition, introduces a co-stimulatory

DOI: 10.54254/2753-8818/21/20230809

endodomain. Several ligands for immunoglobulin (Ig) super-family and TNF receptor family costimulatory receptor have been confirmed by experiments being able to functioning in the enhancement of T-cell expansion and cytokine secretion [13]. The third generation contains multiple co-stimulatory molecules (e.g. CD28). Such designs both allow the CAR T-cells to have a higher affinity to cancer antigens than normal T cells with TCRs and trigger a more rapid, long-lasting and stronger immune response.

4.4. Overcoming resistance to CAR T-cell therapy

Just like many other therapies applied, tumours could grow resistance to CAR T-cell therapy in some circumstances. Just take pediatric ALL for an example. The loss of the CD19 antigen or epitope may be one possible mechanism of therapy failure, thus leading the CAR to lose their target, despite adequate persistence of transferred cells. Combinations of CARs that aim at multiple targets on tumour cells are hopeful to eliminate the single protein loss like this. Another main reason could be failure of expansion and/or persistence of CAR T-cells, probably related to patients' pre-existing T cell quality or other immunosuppressive mechanisms [14, 15]. Researchers trying to bypass these factors have been using precision genome editing like zinc-finger nucleases to successfully develop a 'universal' CAR T-cell line, which collects T cells originally from healthy, allogeneic donors. The universal ones are believed to have the potential to overcome failures associated with autologous T-cell defects such as terminal differentiation [11]. In vivo the immune checkpoint blockade which will be mentioned below with concurrent CAR T-cell therapy may also be effective as a strategy against resistance caused by certain immunosuppressive mechanisms.

5. Immune checkpoint blockade

Just as the methods mentioned above, the existence of suppresser T cells shows the evidence that immune system possesses its own way to attenuate T cell activation, probably in order to prevent abnormal immune reaction to normal somatic cells, which is also called T cell tolerance. We use immune checkpoint to refer to those key nodes (ligands and their receptors) in the inhibitory pathways existing in the immune response. These checkpoints play an important role in normal operation of immune system, including maintaining self-tolerance and regulating the duration and extent of immune responses, primarily to minimize possible damages caused by immune cells against normal tissue [16].

5.1. How ICB works

Some of the tumour cells take advantage of certain immune-checkpoint pathways as their major mechanisms of immune resistance. Some key receptors and their ligands in this process, such as cytotoxic T lymphocyte antigen 4 (CTLA4) , programmed cell death 1 (PD1) and its ligand, PDL1, are used by cancer cells to attenuate T cells for immune escape. Basically most of the immune checkpoints are shown in the pattern of ligand–receptor interactions. Therefore, these checkpoints can be easily blocked by preventing this interaction, produced by competitive binding or recombinant of ligands or receptors, etc [16]. This therapy is called immune checkpoint blockade, or immune checkpoint inhibition, finally resulting in reactivation of T cells against tumours.

5.2. Principle of CTLA4 blockade

CTLA4, as a member of T cell transmembrane protein, has been proved to be an attenuator of T cell activation by Allison et.al [17]. Researchers found that CTLA4 and CD28 competitively bind to the co-stimulatory ligands T lymphocyte activation antigen B7-1 and B7-2, which typically express on APCs. Furthermore, CTLA4 realizes its function that attenuates T cell activation through multiple mechanisms: recruitment of phosphatase to inhibit TCR signal pathway; preventing CD28 binding by changing CD28 localization; blockade of B7 ligands by transendocytosis. Besides direct interference to the signal pathway, CTLA4 can also increase T cell motility and thus reduce the contact of T cells to APCs, resulting in decreased proliferation of them [17].

DOI: 10.54254/2753-8818/21/20230809

Among all immune cells, Treg cells typically express the highest level of CTLA4. Experiments have proved that functional CTLA4 loss downregulates B7 ligands organization of Treg cells, in turn proving that CTLA4 is strongly associated with immune suppression performed by Treg cells. Effective anti-CTLA4 antibodies are able to bind to activated FcγR receptors, thus depleting intratumoural Treg cells while sparing Treg cells in the periphery, finally resulting in an improved Teff/Treg cell ratio within the tumour [17]. Solely use of ligand blockade causes expansion and activation of Treg cells, as a role of negative feedback control, so agents that deplete Treg cells should be applied in combination.

5.3. Principle of PD1 blockade

PD1 is another widely-studied immune checkpoint to be targeted by ICB, with two inhibitory ligands PDL1 and PDL2 as known. PDL1 was previously reported to be an attenuator of T cell activation. In 2002, Chen and his colleagues reported the fact that PDL1 is observed to express in a variety of human cancer tissues. Using a mouse syngeneic tumour model, it has been proved by researchers that PDL1 expression could increase tumour cell proliferation and survival and increase T cell apoptosis, all of which could be neutralized by an anti-PDL1 antibody [18-20]. PDL2 is speculated to play a role in negative regulation of PD1 checkpoint, but it seems confused that solely blockade of PDL2 has not demonstrated any antitumour effects [21].

PD1 mainly expresses during 2 different cellular processes, that is precursor T cells' differentiation into Teff cells or memory cells and activation or reactivation of these T cells. High level of expression of PD1 will cause Teff cells to enter an "exhausted" phase, finally resulting in programmed death. PD1 blockade is proved to be effective in early state of this "exhausted" phase by reactivation, but ineffective in terminal state, which indicate PD1 blockade is "state shift" rather than "state reversion" [17]. PD1 expression is also detected on suppresser T cells (Treg cells), but its function remains for further study [22]. Experiment on mice and observation of an decrease of Treg cells in patients receiving PD1 blockade therapy imply that PD1 may be related to maintenance of Treg cell population [21, 23]. PD1 is also expressed on macrophages and dendritic cells, suppressing their functions and thus supporting tumour growth.

ICB drugs like ipilimumab and nivolumab have already acquire success in treatment for specific cancers. Current focus on ICB is how to enhance its efficacy, mainly by deeper research on molecules and cells related to activation and suppression of immune response. Combination of ICB and other strategies like traditional chemotherapy or newly introduced cell therapy is also promising. Except for PD1 and CTLA4, research on other immune checkpoints such as LAG3 and TIGIT can probably provide more details and availability of ICB efficacy.

6. Conclusion

Immunity works to respond to numerous threats from both outside and inside our body. During the last few decades, researchers have been studying how to make use of our understanding of the fundamentals and principals of immunology to reinforce, regulate or interfere with the immune response, in order to better protect our body from infection or assist immune system eliminating tumour cells. Thanks to these efforts, immunotherapy has made great contribution into human health, and we expect much more from it in the coming future. We use cancer vaccine to present selected antigens and activate DCs, in order to teach immune system to better find and recognize tumour cells. We engineer CAR T-cell to strengthen its' binding to antigens, and transport these stronger 'soldiers' into the immune system. And we use ICB to block the negative regulation of T_{eff} cells from tumour cells, thus reactivating T cell response against cancer.

Future research efforts and clinical research on immunotherapy should be focused on enhancing the efficacy, mostly depending on our further understanding of tumour immune escaping mechanism. The key is to figure out the connections between molecules and mechanisms which closely relate to suppression and reactivation of T cell response, and especially more attention should be directed towards immunosuppressive-related cells and molecules which attenuate immune response to tumours.

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Furthermore, combination of these therapies with other successful strategies such as cell therapy or those more traditional like radiation and chemotherapy has more chances to overcome tumour resistance and enhance the efficacy.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230812

Rabies' proteins' functions and future directions

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Abstract. The rabies virus, which mostly originates from the bite of a sick dog or cat and spreads, kills tens of thousands of people each year, and it is super lethal, with almost no one surviving the infection, s so, everyone is afraid of it. The main body of the rabies virus is bullet-shaped, and each combination of proteins in it is an innate destroyer. From the destruction of cells by the G and M proteins to the transcription of the P and L proteins in concert with each other, the precision, and division of labor of the rabies virus are obvious. Of these, the L protein is the key to the operation of everything, and this paper will explore the destruction of each protein, thereby stopping the spread of RABV. In particular, the possibility that the L protein, unlike rabies vaccines currently on the market, might be a future drug design idea by destroying the structure of RABV after the virus enters the cell. Keywords: Rabies, RABV, Protein Matching, L protein.

Keywords: rabies, RABV, protein matching, L protein.

1. Introduction

Rabies is a harmful neurological disease caused by rabies virus (RABV) infection. Rabies virus causes acute zoonotic infections, mostly in carnivores such as dogs, wolves, and cats. Humans are mostly infected by diseased animals' bites. Clinical manifestations include a characteristic fear of water, wind, pharyngeal muscle spasms, and progressive paralysis. There is almost no chance of survival if clinical signs appear [1]. Rabies vaccination is given after being bitten by an animal that carries the rabies virus. This event is done so that the antibodies are present in the body for a long time. This way, when the actual disease-causing rabies virus appears, the antibodies can quickly destroy it before it can multiply. There is no effective treatment for rabies, but exploring the amino acid sequence of the L protein might be helpful.

The rabies virus is transmitted to humans and animals through the saliva of a sick animal and then enters the infected body through a wound. Invasion of the neuromuscular junction followed by interaction with nicotinic acetylcholine receptor proteins spreads the infection to other peripheral nerves. Subsequently, the extended infected nerve spreads to the central nervous system. This event results in damage to neurons in the brainstem and cerebellum. In the gray matter of the brain, the viral nucleoshell is expelled from the endosome and transported along microtubules to form new virosomes or secondary viral factories [2]. It contains five proteins: polymerase (L), matrix protein (M), glycoprotein (G), and nucleoprotein (N). In figure 1, the genes for these five proteins line up after the 58 nt leader sequence in front of Q, in the order 3′-N-P-M-G-L-5′. After these are followed by the 57-70 nt trailer [3]. The RABV divided into two parts: the surfing envelope and the RNP. Nucleoprotein. The surrounding envelope is composed of knob-like spikes of glycoprotein, while the RNP contains Nucleoprotein [4].

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DOI: 10.54254/2753-8818/21/20230812

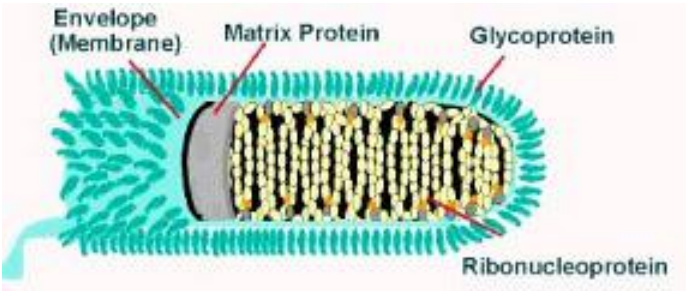

Figure 1. shows the rabies virus, which is coated in spike-like glycoprotein peplomers that are 10 nm in size [4].

Glycoprotein (G) catalyzes and causes membrane fusion, which enables the virus to enter the groundplasm and cause infection. Succeeding full entrance into the cell, the P protein helps the L polymerase transcribe the genome of the virus to create circulating viral proteins [5]. The L protein produces five strands of mRNA and one strand of positive RNA from the first negative-stripped RNA. These five mRNA strands are translated into the relevant proteins on ribosomes that are floating in the cytoplasm. Post-translational modifications are necessary for some proteins. When there are sufficient viral proteins present, viral polymerase makes negative-stranded RNA from positive-stranded RNA templates. These averse strands will go to the inner cell membrane in a combination with the protein. The newly created the pathogenic particle's outer envelope is made up of a G protein that is incorporated in the membrane and surrounds the protein complex. When the viruses reach a certain number of replications, they start to bind to acetylcholine receptors at the neuromuscular junction and cross the axon of the nerve cell by retrograde transport. In this section, their P protein interacts with proteins in the cytoplasm of the nerve cell, causing the virus to swiftly enter the brain's nervous system once it reaches the cell body, replicating in motor neurons, and eventually reaching the brain. L protein is essential to the way that RABV is transmitted. It is the biggest structural protein of the RABV and necessary for the synthesis of every viral protein, including the virus itself.

The negative-stripped RNA is converted into a positive strand of RNA by the L protein from the original negative-stripped RNA. On free ribosomes in the cytoplasm, these five mRNA strands are translated into the necessary proteins. Some proteins require post-translational changes. Viral polymerase converts positive-stranded RNA templates into negative-stranded RNA when there are enough viral proteins present.

2. About G protein

Treatment for RABV generally begins with G protein. In figure 2, there is a type I glycosylated protein and a trimer consisting of 524 amino acids. This polypeptide contains 524 amino acids, including 19 amino acids in the signal sequence. Arginine at locus 333 is crucial in the virulence of RABV. This is associated with neuroinvasiveness and the ability to spread across synapses, enabling the virus to spread faster in the nervous system. The glycoprotein is a significant factor that determines whether or not RABV can enter and infect [6]. In order to induce endocytosis of the virion, the virus binds to the host cell's receptor via the G protein. The acidic pH in the endosome causes conformational changes in the glycoprotein trimer, which results in membrane fusion. Low pH induces different conformational states in elastovirus glycoprotein during membrane fusion. Blister virus glycoproteins can be classified by their structural features as pre-fusion, early intermediate, late intermediate, and post-fusion. While RABV virus glycoproteins usually exist as trimeric spikes on their surface, the RABV virus glycoproteins are mainly trimeric spikes. In solution, however, it mainly exists as a monomer, not as a trimer [7]. Various in vitro experiments have shown that the muscular form of the nicotinic acetylcholine receptor (nAChR) binds glycoprotein, facilitating rabies virus entry [8]. That is the reason why the investigation wants to study glycoprotein because if it can cut the beginning of the infection.

DOI: 10.54254/2753-8818/21/20230812

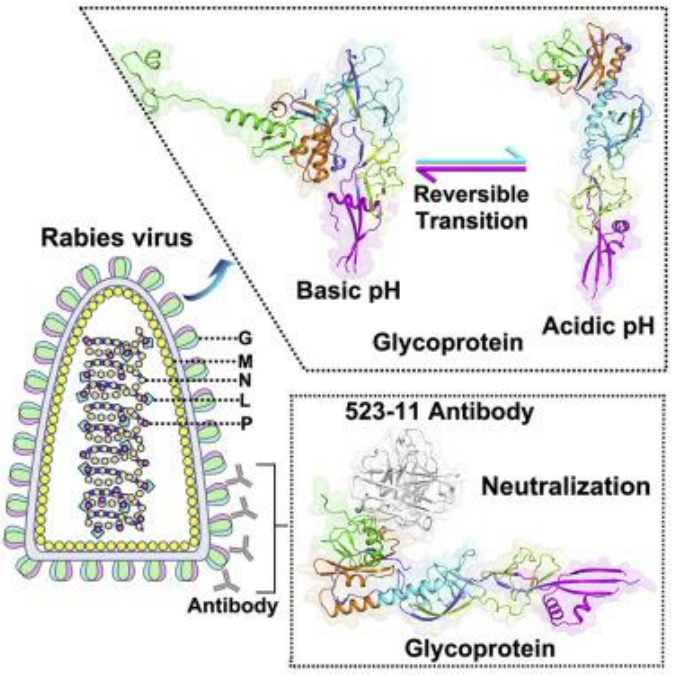

Figure 2. Analysis of the rabies virus glycoprotein's structure [7].

3. About M protein

The M protein is necessary for viral assembly, acts as a matrix and is a major component of the entire virion. As a pathogenicity determinant, it determines the virus's assembly and germination. Cell membrane disruption occurs due to the amino acid at position 95 of the M protein [8]. The previously mentioned viruses generate nucleocapsids when they enter the gray matter of the brain. this ability to bind and possibly coalesce nucleocapsids exists in the M protein. It can also help the G protein to enter [9]. While M proteins interact with RNP and G proteins and play an important role in recruiting RNP to host cell membranes and in outgrowing viral particles, they also interact with other proteins. It is also possible for M proteins to act synergistically with the JAK-STAT pathway in the absence of G proteins to regulate the pathway [10].

4. About N protein

Nucleocapsid proteins are proteins. The N protein always encases genomic RNA. N proteins congregate with ribonucleic acid to inhibit nucleases [11]. Both N-RNA complexes include single-stranded RNA with random sequences and are organized as loop assemblies. It aids in wrapping and folding virus particles. It has been established that the N protein's pathogenicity determinants, amino acids 273 and 294, block the generation of IFN, which in turn affects the ability of viruses to enter the brain. This shows that by enabling successful viral transmission, the RABV N protein contributes significantly to avoiding the brain's instinctive immune system reaction [11]. N proteins are equally as important as the G protein in enabling membrane fusion to avoid innate immune system components during vivo. N proteins play a crucial role. They influence the ability of in vivo proteins to inhibit the host antiviral response. Mainly, by facilitating efficient virus transmission [9].

5. About P&L protein

It was discovered that the NPYNE sequence is necessary for the interactions between the L and P proteins, and this region is indicated in light orange and pink in the figure 3. The binding domain of this L-protein sequence is at the C-terminus of P-protein-L. Because L proteins require the interaction with their important cofactor P proteins, the activity of L proteins as RdRp all depends on this region [17].

DOI: 10.54254/2753-8818/21/20230812

Scientists find that the L protein is the largest structural protein, consisting of 2127-2142 amino acids, and is an enzyme complex of approximately 244 kDa that is fundamental for producing all RABV proteins, including its own, because it functions as a dependent RNA polymerase (RdRp). In the figure 4, L protein can be classified into three macromolecular structures: Glycoprotein, RVA122 Fab Light Chain, and RVA122 Fab Heavy Chain. The L protein has a small receptor molecule called NAG [12]. Since the L protein is associated with all other RABV genes, inhibiting the L protein from it could be considered. L proteins are of significance for viral binding, replication as well as transcription [10]. In the cytoplasm, negative-stranded RNA is translated into five strands of mRNA and one strand of positive RNA. By free ribosomes located in the cytoplasm, all five of these mRNA strands are translated into proteins. It is noteworthy that in this process, the interplay of L and P proteins is required for completion. Despite the lack of information on the three-dimensional structure of the L protein, RABV's L protein is significantly similar to the fully characterized L protein of VSV in amino acid sequence, structural domain structure, and enzymatic function. This was then compared with the structure determined by cryo-electron microscopy of the VSV L protein. Although the L protein binding site could not be determined, it was seen that if started from the RABV P protein, it stimulated the initiation of transcription, and the extension mediated by the L protein [13].

The P protein is a regulatory and non-catalytic polymerase cofactor that aids in viral transcription and replication [14]. The endosomal structure requires the development of dimerized structural domains of P proteins. This stretch, which is mediated by the P and L proteins, is found in the residues of the P protein. A significant loss in the carboxy-terminal region of P does not affect its ability to interact with the L protein. The trials and the outcomes do not necessarily prove that L proteins need P proteins. However, the P protein and L protein cannot be separated. The former also binds to the soluble protein N to prevent it from wrapping around non-genomic RNA and remains on the N-RNA template to keep the RNA polymerase L stable. The P protein contains two N-binding sites. As a result, the P protein's N and L protein binding sites do not overlap. P proteins can behave as replication factors or transcription factors depending on whether they are paired with L proteins or N proteins. The P protein binds to the N protein, which in turn directs the G protein, so long as it can block the L protein while also destroying the P protein, which in turn destroys the N and G proteins [15].

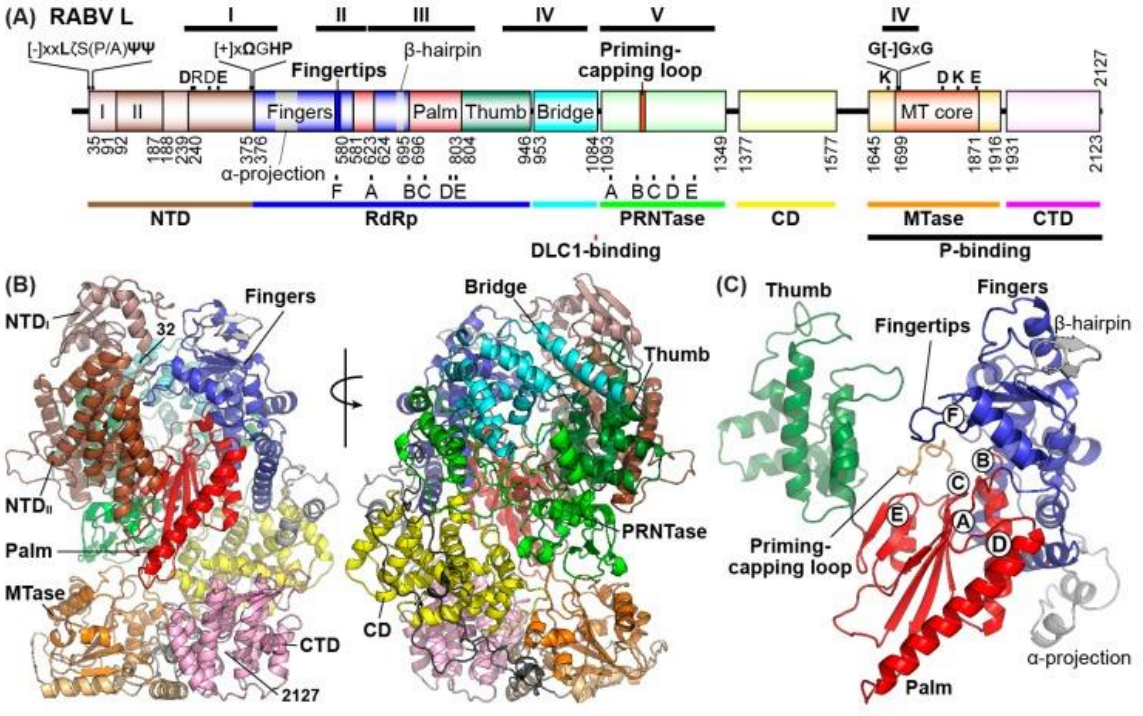

Figure 3. The L protein of RABV [13].

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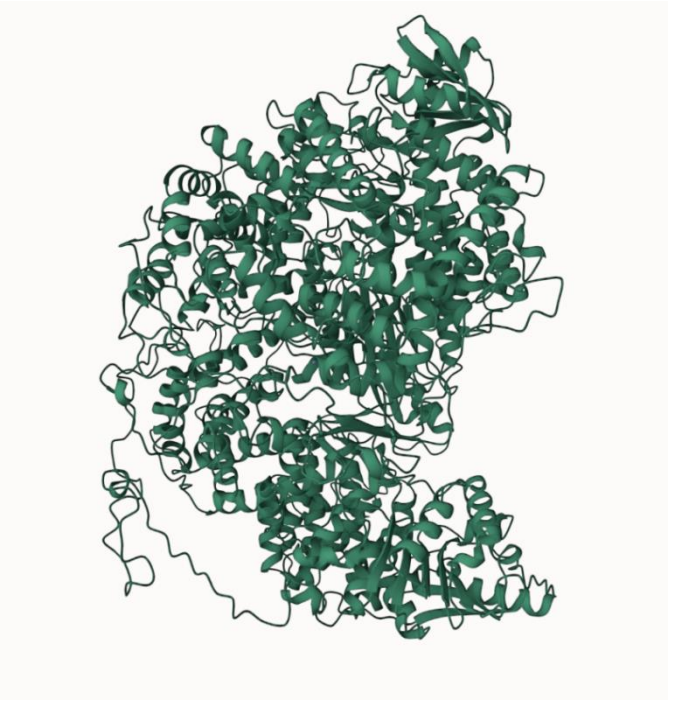

Figure 4. Structure guessing of L protein in RABV [16].

The graphic above displays the expected L protein sites for the RABV based on VSV. The numbers indicate the start and end points of the structural and sub-structural domains of amino acid residues. The picture shows the preserved region (I-VI), the RdRp pattern (A-F), and the PRNTase pattern (A-E). The L gene-deficient RABV (Nishi-L/Nluc) was developed by Kento Nakagawa et al. through reverse genetics. NanoLuc luciferase was used to transfect cultured neuroblastoma cells in order to create L protein. To determine the functional importance of the highly conserved L protein region between positions 1914 and 1933, back-complementation was employed with mutant L proteins. It was discovered that the NPYNE sequence at positions 1929 to 1933 is necessary for the interactions between the L and P proteins, which is also shown on the graph in light orange and pink. The binding domain is at the C-terminus of P protein-L. The interaction of the L protein with the P protein, and therefore the L protein's activity as RdRp, depend on this area [17].

6. Conclusion

The proteins of rabies virus have a very close cooperation: the G protein, assisted by the N protein, serves as the main destructive protein structure of rabies. The main body of the virus is the M protein, and the critical viral transformation depends on the cooperation of L and P proteins. The important sites of cooperation between the P and L proteins are at amino acids 1929 to 1933 of the L protein of RABV, which can be designed as a drug target for direct destruction not in the external tissues but in the interior. In other words, starting with the L protein may contribute to new advancement in treating rabies in the future.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230813

Binding modes in ligand-docked hepatitis B virus core protein simulated by a Monte Carlo method

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Abstract. Hepatitis B virus core protein (HBV Cp) is closely involved in the viral assembly, nuclear functions, compartment for reverse transcription, and intra-cellular trafficking. Therefore, modulation of HBV Cp assembly is a promising method to control HBV infection in both preclinical and clinical studies. In this paper, two ligands of HAP18 and AT-130, as representations of heteroaryldihydropyrimidines and phenylpropenamides, have been chosen. Their Binding modes and conformations docking with the HBV Cp have been simulated by a Monte Carlo method. Strong polar contact in HAP18-bound and relatively weak interaction dominated by the Lennard-Jones potential in AT130-bound have been observed, respectively. Although different binding modes result in different assembly behaviors of HBV core protein, both of them strongly influence the quaternary structure of the Cp assembly, changing the spatial relationship between dimers, and inducing noninfective Cp misassembly. The simulation is expected to be helpful to get some insight into the antiviral mechanism of HBV Cp assembly modulation.

Keywords: hepatitis B virus core protein, HAP18, AT-130, capsid assembly modulation, binding modes.

1. Introduction

According to the World Health Organization, approximate 260 million individuals are infected by hepatitis B virus (HBV) worldwidely and 0.9 million die from HBV-related liver cancer or cirrhosis every year [1]. Drugs, such as the direct-acting nucleotide analogues interfering the immunomodulating interferon alpha and the viral reverse transcriptase, have been used to control the progression of the disease, but they are being hindered in clinics due to their noneffective curative and severe side effects [2].

The DNA of HBV is protected by an icosahedral capsid self-assembled by core protein (Cp) dimers. The capsid plays important part in the viral assembly, regulation and completion of reverse transcription, intra-cellular traffificking, and nuclear functions [3]. Antiviral drugs targeting the Cp have potentials to inhibit assembly of viral particles, viral DNA replication and cccDNA synthesis. Binding small-molecule ligands (core protein allosteric modulators (CpAMs)) to the heteroaryldihydropyrimidine pockets of the HBV capsid can induce Cp dimer misassembling to increase the capsid assembly rate, destabilize the HBV core protein, and result in either aberrant or empty, nonfunctional capsid particles [4,5]. It consequently blocks viral pgRNA and pol packaging into the nucleocapsid and subsequent viral DNA replication. In recent years, as a promising treatment,

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DOI: 10.54254/2753-8818/21/20230813

modulation of virus assembly has been attracted much attention, and a variety of novel capsid assembly modulators have been reported [6].

2. Methodology

The binding of ligands to large protein receptors is central to numerous biochemical processes. Computer-aided simulation is a powerful tool to predict the binding modes with an acceptable accuracy in the drug design. It is well established that the protein docked by ligand must indicate a structure with the globe minimum free energy. In order to calculate such state, basically, two types of algorithms are applied [7]. One is Molecular Dynamics (MD), which involves in solving the Newton's equations of motions. However, all degrees of freedom need to be considered in MD. If the system contains two sets of degrees of freedom whose characteristic time is far apart, the method will be in dilemma. Furthermore, the calculation results extremely depend on the initial conformation of the system and usually obtain a local minimum energy state, because the molecule trajectory is easily trapped on the rugged hypersurface of the protein. Another one is Stochastic Dynamics (SD), which involves the calculation of the total energy at each possible docking position. Thus, the simulation results of SD no longer depend on the initial conformation of the ligand-receptor system because that a simple energy function is used and the energy barriers on the hypersurface are simply stepped over. Comparing to MD, SD is much efficient because the amount of computation can be reduced attributed to the fact that some degrees of freedom can be replaced by a random force. In this paper, we have simulated a homodimer of HBV Cp docked by two CpAMs with SD technique combining a Monte Carlo search method, which applies random moves of the ligand molecule in the gridded calculation area and accepts/rejects the move based on a Metropolis criterion. The conformation of the docked ligand has been analyzed.

3. Results and discussion

The structure of Hepatitis B virus core protein (PDB ID: 1QGT) as the receptor of this simulation was provided by the Protein Data Bank as reported by Leslie et al. [8]. As shown in Figure 1, the HBV capsid with a icosahedral symmetry (T=4) is composed of 60 core protein homodimers. Four Cp monomers which differ slightly from each other in quasiequivalent environments form one basic functional unit of HBV Cp. (Figure 1(b)). Schrödinger 2021-2 was used to carry out the virtual screening. During the preprocessing, missing hydrogens were added to the structure, and the structure was then energy-minimized using OPLS 4. Two small molecule ligands (HAP18 and AT-130), representing heteroaryldihydropyrimidines and phenylpropenamides, respectively, were chosen as CpAMs. Sdf files of the ligands were imported to the workspace. Using LigPrep function in Schrödinger 2021-2, the 3D conformations of two ligands in human plasma of PH=7.4 was simulated with OPLS4 (as shown in Figure 2). It is found that the bonds rotate angles to meet the requirement of the minimum energy state. The Cp receptor was considered as a rigid entity, while the ligands were flexible (the TORSDOF freedom of HAP18 is 7 and that of AT-130 is 6). A cubic grid box with side length of 47.25 angstrom was constructed, as big as possible to cover the whole dimers. A random move, including the move of the ligand mass center along the protein hypersurface in the grid box and the rotation of the bonds, is applied to the ligand. Subsequently, the energy comprising five contributions: Lennard-Jones potential; hydrogen bond; coulombic electrostatic potential; term related to the number of sp3 bonds; and a desolvation term. Finally, the minimized structures are accepted based on the Metropolis acceptance criterion. In the calculation process, step of the random move depends on the curvature of the protein hypersurface assessed by the second derivative of the energy function. Large steps are attempted in areas of small curvature and small steps are attempted in those of large curvature. This Monte Carlo search technique is highly efficient.

DOI: 10.54254/2753-8818/21/20230813

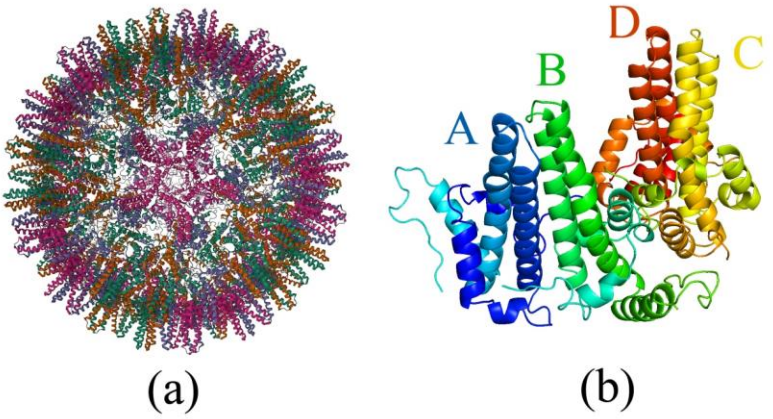

Figure 1. Structure of HBV capsid. PDB ID: 1QGT obtained from the Protein Data Bank as reported by Leslie et al. [8]. (a) The quaternary structure of an HBV capsid. (b) Close-up of a homodimer composed of AB dimer and CD dimer, or chain A-blue, chain B-green, chain C-yellow and chain D-brown.

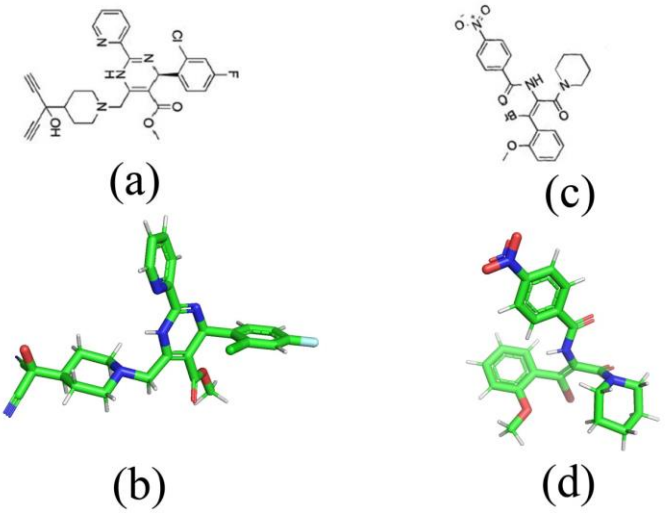

Figure 2. Ligands of HAP18 (a) and AT-130 (c) and their 3D conformations in human plasma of PH=7.4 simulated with OPLS4, (b) HAP18, (d) AT-130, the different atoms are colored: carbon-green, oxygen-red, nitrogen-blue, and hydrogen-gray.

Figure 3 indicates that there are two binding sites on the Cp homodimer for the HAP18 docking. One is located between chain A and chain B (Figure 3(a)), while another one binds to the hydrophobic pocket on the dimer-dimer interface (Figure 3(b)). Two polar contacts between oxygen and nitrogen atoms are observed, inferring a strong ligand-protein interaction. Such binding enhances the stability of the intra-protein interactions, increases the rate of HBV core protein assembly, and induces the formation of large, regular complexes as reported by Bourne C. et al. [9]. Figure 4 shows the AT130-bound core protein. The binding sites are located the dimer-dimer interfaces (between chain A and chain D for docked AB dimer (Figure 4(a)) and between chain B and chain C for docked CD dimer (Figure 4(b))). The AT130-protein interaction is much weaker than that of HAP18-bound, no polar contact is discovered. The Lennard-Jones interaction results in bonds rotation so that AT130 molecule form a conformation fitting the rugged protein hypersurface. It has been found that both HAP18 and AT-130 binding to HAP pockets can lead to quaternary structural changes in the nucleocapsid, but HAP18 induces a much more significant quaternary rearrangement than AT-130 due to the stronger interaction

DOI: 10.54254/2753-8818/21/20230813

between HAP18 and receptor. AT-130 can also compensatively cause tertiary structural changes during nucleocapsid assembling [10]. Such effects should be related to the binding mode induced by two ligands.

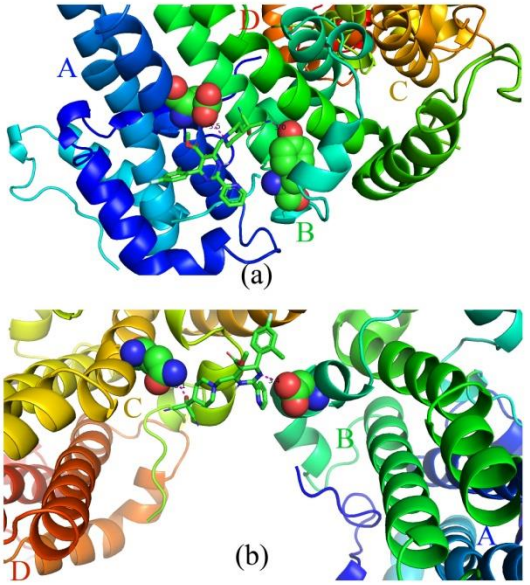

Figure 3. Simulated optimal binding modes and conformations of (a) HAP18 with AB dimer and (b) with CD dimer, respectively. Oxygen-Nitrogen polar contacts are indicated with red dash lines. Biding sites appear at chain-chain interface (a) and dimer-dimer interface (b).

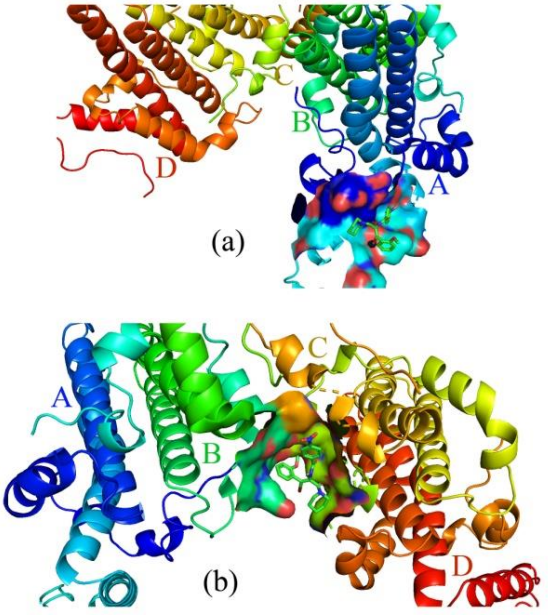

Figure 4. Simulated optimal binding modes and conformations of (a) AT-130 with AB dimer and (b) with CD dimer, respectively. The binding sites are located the hydrophobic pockets on the dimer-dimer interfaces: (a) between chain A and chain D for docked AB dimer and (b) between chain B and chain C for docked CD dimer.

DOI: 10.54254/2753-8818/21/20230813

4. Conclusion

In conclusion, chosen ligands of HAP18 and AT-130 were chosen to represent two different types of CpAMs and to dock with the HBV capsid homodimer. The binding modes and conformations of them have been simulated with a Monte Carlo method, which is stochastic and ergodic but computational time saving. It has been found that HAP18 prefers to a polar contact with the HBV capsid, binding to either the interchain site at the AB dimer or the hydrophobic pocket on the AB-CD dimer interface. AT-130 prefers to a relatively weak Lennard-Jones interaction, binding to the hydrophobic pockets at the dimer-dimer interface. The difference in the interactions between ligands and receptor has resulted in different effects on the conformation of dimer assembly. Both of them lead to quaternary structural changes in the nucleocapsid, and then altering the spatial relationship between dimmers, but HAP18 induces a much more significant quaternary rearrangement than AT-130 due to the stronger interaction. AT-130 can compensatively cause tertiary structural changes during nucleocapsid assembling. These changes are large enough to cause noninfective Cp misassembly. The simulation results have indicated that the bindings of HAP18 and AT-130 are not competitive at Cp hypersureface. This paper therefore proposes that multiple ligand-binding is worth trying to improve the effects of CpAMs.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230819

Research on the parasomnia's classifications, symptoms and treatments

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Abstract. Parasomnia is a neurological disorder that refers to abnormal behavior during sleep. This disease has received increasing attention and research over the past few decades. In the 1950s, the symptoms of parasomnia were first described and further studied. This paper describes the three classifications of parasomnias, and details the symptoms and treatment options for these three classifications. By reading and analyzing the previous literature, it can be concluded that the types of parasomnia can be roughly divided into circadian dysrhythmia sleep disorder, sleep apnea syndrome, and restless leg syndrome. Circadian dysrhythmia sleep disorder is characterized by late sleep, insufficient or excessive sleep duration, resulting in daytime fatigue, a lack of concentration, and other symptoms. The treatment method is light therapy. The main symptom of sleep apnea is that breathing stops during sleep, resulting in a lack of oxygen supply. The treatment is to improve the maxillofacial structure through surgery to reduce the problem of dyspnea. The most obvious symptom of restless leg syndrome is discomfort and unbearable numbness in the lower legs and feet while sleeping. There are three ways to treat this condition. A more non-pharmacological treatment is to do some exercise to reduce the discomfort of the leg muscles, a medical treatment is to give the patient levodopa, and if the syndrome is caused by a lack of iron in the body, iron supplementation is sufficient.

Keywords: clock gene, circadian dysrhythmia, restless leg syndrome, levodopa, sleep apnea syndrome.

1. Introduction

Sleep is an integral part of human life and has a significant impact on both physical and mental health. However, some people experience sleep differently from normal sleep patterns. They may wake up during the night and have trouble falling asleep, or they may feel sleepy during the day and have trouble staying awake. These abnormal sleep experiences are called parasomnias. Parasomnia refers to a series of abnormal phenomena that occur during the normal sleep cycle. Parasomnia can be caused by a variety of factors, including genetics, obesity, gender, age and so on. These factors may lead to decreased sleep quality and affect daily life and work performance. Therefore, the purpose of this paper is to make people understand the types, symptoms and treatment methods of parasomnia, so as to improve their understanding of it.

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DOI: 10.54254/2753-8818/21/20230819

2. Analysis of parasomnia's classifications and symptoms

In this chapter, parasomnia is divided into three categories (i.e., circadian dysrhythmia sleep disorder, sleep apnea syndrome, and restless leg syndrome), and describes the three different forms of symptoms.

2.1. Circadian dysrhythmia sleep disorders

Circadian dysrhythmia refers to a sleep disorder in which the body's biological clock is out of sync with the 24-hour day-night cycle, resulting in abnormal sleep-wake patterns. The biological clock refers to the natural biological rhythm inside the human body, which is based on periodic changes in the natural environment, such as light, temperature, food intake, etc. Circadian rhythms refer to the body's daily changes in a 24-hour cycle. Circadian dysrhythmia usually refers to the mismatch between the biological clock and the circadian rhythm, which leads to a series of symptoms: Daytime fatigue and sleepiness: due to poor sleep quality, patients often feel tired and drowsy during the day, affecting work and life. Insomnia: Patients have difficulty falling asleep or waking up multiple times during the night, resulting in poor sleep quality. Early awakening: Patients have difficulty falling back asleep after waking up in the morning, resulting in insufficient sleep time. Emotional instability:Sleep disorders and circadian rhythm disorders may lead to emotional instability, irritability, anxiety, depression, etc. Lack of concentration: Insufficient and low-grade sleep can affect patients' cognition and concentration, resulting in reduced learning and work efficiency. Altered appetite: Disturbed circadian rhythms may affect the patient's appetite and digestive system, leading to problems such as an upset stomach and decreased or increased appetite.

The normal operation of circadian rhythm is determined by a core set of CLOCK genes, including the PRE gene family (including PRE1, PRE2 and PRE genes), the CRY gene family (including CRY1 and CRY2 genes), the CLOCK gene and the BMAL1 gene [1]. These genes encode a series of clock proteins and regulate the circadian clock cycle through complex interactions. Therefore, abnormal and dysregulated circadian rhythms are associated with genetic factors. According to Mansour et al, Nievergelt et al and Wellcome Trust Case Control Consortium, circadian rhythm disturbances are associated with variations in clock genes [2]. Because these genes interact through a complex regulatory network to control the body's circadian rhythm. And when the expression of these genes is mutated or mutated, the circadian rhythm becomes dysregulated, leading to a series of sleep disorders and physiological diseases. The etiology of such parasomnia is related to genetic factors.

2.2. Sleep apnea syndrome

One type of parasomnia is Sleep Apnea Syndrome (SAS), which is a common sleep disorder[3]. The main feature is apnea during sleep, which results in insufficient oxygen supply and causes a series of health problems. Sleep apnea syndrome is divided into two main types, namely obstructive sleep apnea (OSAS) and central sleep apnea (CSAS). Obstructive sleep apnea is caused by a partial or total blockage of the upper airways, resulting in a pause in breathing. Central sleep apnea is caused by damage or disturbance to the respiratory control center, resulting in the suspension of breathing [4]. Obstructing sleep apnea is characterized by apnea and snoring caused by partial or total obstruction of the upper airway, and symptoms include frequent nighttime awakenings, drowsiness, daytime fatigue, and inattention [4, 5]. Central sleep apnea is characterized by apnea due to damage or disturbance of the respiratory control center. Its symptoms are similar to those of obstructive sleep apnea, but are often accompanied by cardiovascular and neurological problems, such as hypertension, arrhythmia and cognitive impairment. Both types of sleep apnea can lead to decreased sleep quality, which in severe cases can affect quality of life and health.

Obesity factors, age, and gender can all influence the incidence and severity of sleep apnea syndrome.

First, obesity is one of the most common risk factors for sleep apnea syndrome. Excessive fat accumulation can cause the tissue in the throat to relax, leading to obstruction or narrowing of the airways, which can affect breathing. Studies have shown that obese people are four to five times more likely to develop sleep apnea. In addition, studies have found that the symptoms of sleep apnea syndrome can be significantly improved by weight loss [6].

DOI: 10.54254/2753-8818/21/20230819

Secondly, age is also an important factor affecting sleep apnea syndrome. With age, muscle tone and the elasticity of the respiratory system decrease, which can easily cause apnea. Studies have found that the incidence of sleep apnea syndrome increases with age, especially in men [7].

Finally, gender is also associated with the incidence and severity of sleep apnea syndrome. Men are more likely to suffer from sleep apnea than women, and the reason may be related to the physiological structure of the male throat and neck, which is more likely to cause airway obstruction or narrowing. In addition, hormones have an impact on respiratory function in both men and women. Among them, male hormones have a certain impact on the collapse of the upper respiratory tract [8], which may indirectly lead to the generation of obstructive sleep apnea.

2.3. Restless legs syndrome

Restless legs syndrome (RLS) [9, 10] is a neurological disorder characterized clinically by discomfort or unbearable sensations of numbness, tingling, or tearing in the lower limbs, especially the calves and feet, but also involving the thighs and buttocks. These symptoms become more pronounced at night. As a result, the disease can cause symptoms such as difficulty falling asleep, lack of deep sleep, lack of concentration during the day and irritability, all of which seriously affect the quality of life of patients. Some studies have shown that the prevalence of RLS is age-related, with increasing age increasing the incidence of RLS, and that RLS is more common in women than in men [11]. In addition, the syndrome is associated with periodic limb movements (PLMS), which are also present in about 80% of RLS patients. PLMS, which can be diagnosed by polysomnography, is characterized by unilateral or bilateral limb movements that may reduce the quality and quantity of sleep at night and disrupt the sleep of bed-mates. Therefore, treating RLS and PLMS is very important to improve sleep and quality of life [12].

Genetic factors are being studied as a possible cause of restless legs syndrome. Therefore, the twin experiment is an appropriate research direction to prove the existence of this factor. In Ondo's study, it was found that there was a strong concordance of restless legs syndrome in monozygotic twins and a low concordance in dizygotic twins [13]. In another study, a mother and two of her identical twin children were also found to have RLS [14]. One conclusion we can draw from these two sets of studies is that there is a genetic link to restless legs syndrome.

3. The treatment of parasomnia

3.1. The treatment of circadian dysrhythmia disorders

Light therapy is a common treatment for sleep disorders with circadian rhythm disorders. This treatment is mainly achieved by acting on the retina of the human body. Light-sensitive pigments (photoreceptors) in the retina sense light and send messages to the brain that affect the sleep clock. Specifically, when the human eye is exposed to bright light, the photopigment is stimulated, and the signal to the pituitary gland that regulates melatonin secretion is reduced, thus reducing melatonin levels. In contrast, when people are in the dark, the pituitary gland releases regulatory signals that increase, promoting the secretion of melatonin, so that melatonin levels rise. Therefore, light therapy can help patients regulate their circadian clock and sleep cycle by regulating the level of melatonin secretion through the intensity and duration of light [15].

3.2. The treatment of sleep apnea syndrome

The surgical treatment of sleep apnea syndrome mainly includes laryngeal surgery and maxillofacial surgery. Larynx surgery includes tonsillectomy, palatal obturation resection, tongue base resection, etc. These operations mainly treat upper respiratory tract obstruction factors, and improve respiratory tract patency by reducing obstruction sites. Maxillofacial surgery includes maxillofacial bone advancement surgery, mandibular advancement surgery, etc. These operations mainly treat maxillofacial deformity and other factors, and reduce upper respiratory tract obstruction by improving the maxillofacial structure. This treatment is often used to treat sleep apnea. In the report of Olsen et al. [16], six patients with obstructive sleep apnea were presented. All six patients underwent surgery to correct airway obstruction

DOI: 10.54254/2753-8818/21/20230819

and relieve sleep apnea, thus avoiding permanent tracheotomies. These cases also show that surgical treatment can effectively help patients with obstructive sleep apnea relieve their pain.

3.3. The treatment of restless leg syndrome is divided into two categories: non-drug treatment, drug treatment and supplement nutrients.

3.3.1. Non-drug therapy. Restless leg syndrome is a common neurological disorder, that is usually treated with drugs to relieve symptoms. However, non-drug therapies can also be used as adjunctive treatments to help reduce the symptoms of restless leg syndrome and improve quality of life. Non-drug treatments include exercise, massage, physical therapy, etc.

Exercise is a very effective non-drug treatment, and proper exercise can ease the discomfort of restless leg syndrome, thus reducing symptoms. At the same time, appropriate physical exercise can also increase the duration of deep sleep and reduce the duration of light sleep. In addition, exercise can also reduce anxiety, depression and other negative emotions, promote physical and mental health, and further improve sleep quality [17]. The type of exercise can be walking, jogging, swimming, yoga, etc., but it should be noted that the exercise time should not be too late, so as not to affect sleep.

Massage is another non-drug treatment that reduces the symptoms of restless leg syndrome by relieving muscle fatigue, improving blood circulation, and calming the nervous system. The method of massaging calf muscles is usually used, and massage services provided by professionals can be selected, or a simple massage can be carried out by an individual.

Physical therapy is another non-drug treatment method, and commonly used physical therapy includes hot and cold compresses, electric therapy, etc. A hot compress can relieve muscle fatigue and relax muscles, thus reducing the symptoms of restless leg syndrome. A cold compress can relieve muscle pain and swelling. Electrotherapy is a treatment method that improves nerve and muscle function through electrical stimulation, and reduces the symptoms of restless leg syndrome by stimulating the nervous system.

In conclusion, non-drug therapy can be used as an auxiliary means for the treatment of restless leg syndrome, which can not only reduce symptoms, but also improve physical and mental health, which is conducive to improving life.

3.3.2. Drug treatment. Levodopa is a drug used to treat restless legs syndrome [18]. L-dopa is converted into dopamine in the body, which is a neurotransmitter that helps reduce the symptoms of restless legs syndrome. Levodopa is an oral medication, usually taken before bed, and its effects can last for about four hours. However, it should be noted that long-term use of levodopa may lead to a gradual weakening of its efficacy, so the dose needs to be gradually increased. In addition, long-term use of levodopa may cause side effects, such as nausea, vomiting and dizziness. Therefore, it is necessary to follow the doctor's advice and pay attention to the dosage and timing of drug use to ensure the safety and effectiveness of drug therapy. In addition to medication, some non-medication treatments, such as regular exercise, changes in the sleeping environment, and emotional stress relief, can also help reduce the symptoms of restless leg syndrome.

3.3.3. Supplement nutrients. Iron supplementation can improve restless leg syndrome, so it is the primary goal of iron supplementation therapy to observe the index of iron storage in the human body. The most appropriate method is to determine the iron content of the human body by serum ferritin, transferrin, and transferrin saturation [19]. It has also been shown that iron depletion can make RLS more severe [20, 21]. Iron supplements include oral gluconate, which can cause gastrointestinal side

DOI: 10.54254/2753-8818/21/20230819

effects including nausea, abdominal pain and constipation. Due to the existence of side effects, it is necessary to control the dosage of oral supplements to avoid iron overload [22].

4. Conclusion

The causes of parasomnia are complex, involving genetics, obesity, gender, age and other factors. Therefore, the treatment of parasomnia is also challenging. At present, the treatment of parasomnia includes surgical treatment, behavioral treatment and drug treatment. Behavioral therapy includes daily physical activity, and drug therapy includes the use of levodopa. It should be pointed out that there are some limitations to the current treatment of parasomnia, such as the possible side effects of medication. Therefore, the treatment of parasomnia needs further exploration and improvement.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230820

A lipid-based LMP2-mRNA vaccine to treat nasopharyngeal carcinoma

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Abstract. This paper focuses on developing a lipid-based vaccine targeting nasopharyngeal carcinoma (NPC), a highly severe and invasive epithelial malignancy associated with the Epstein-Barr virus (EBV). The researchers selected EBV latent membrane protein 2 (LMP2) as the preferred antigen for the vaccine. They synthesized full-length LMP2 using an in vitro transcription method and encapsulated it into cationic liposomes based on (2,3-dioleacyl propyl) trimethylammonium chloride (DOTAP) to create the mRNA vaccine (LPX-mLMP2). Cell assays in the study demonstrated that antigen-presenting cells efficiently took up LPX-mLMP2 and expressed LMP2. This led to the formation of peptide-major histocompatibility complexes (pMHC) for presentation. Moreover, the proliferation of antigen-specific T cells at the tumor site indicated the promising potential for mRNA vaccines in combating virus-induced cancers, such as NPC. The researchers concluded that the newly developed mRNA vaccine encoding the antigen offered advantages in the context of NPC and highlighted the attractiveness of mRNA vaccines as candidates for cancer immunotherapy.

Keywords: LMP2-mRNA, Nasopharyngeal carcinoma, Epstein–Barr virus.

1. Introduction

Nasopharyngeal carcinoma (NPC) is the most frequently occurring cancerous tumor in the head and neck region and is primarily found in East and Southeast Asia. Global statistics from 2020 indicate 133,354 newly diagnosed cases and 80,008 deaths attributed to NPC [1]. The primary treatment approaches for NPC involve radiation therapy and chemotherapy. These treatments have shown promising outcomes for NPC patients in the early and intermediate stages of the disease. However, local recurrence and distant metastasis often lead to treatment failure and reduced survival rates.

The Epstein-Barr virus (EBV) is a commonly found pathogen, and it is estimated that more than 90% of adults worldwide have been infected with EBV [2]. EBV infection is strongly linked to Nasopharyngeal Carcinoma (NPC) and is responsible for most cases. Certain latent EBV antigens possess strong immunogenic properties and can stimulate the activation of cytotoxic T lymphocytes (CTLs) that target specific antigens. These CTLs have become the focal point of immunotherapies

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DOI: 10.54254/2753-8818/21/20230820

designed to combat EBV-associated malignancies. Therefore, there is promise in utilizing therapeutic EBV vaccine strategies to treat NPC. Some of these strategies have already undergone clinical trials, such as dendritic cell (DC)-based vaccines [3] and recombinant viral vector-based vaccines [4, 5]. DC-based EBV vaccines have demonstrated effectiveness and safety in individuals who respond to them. However, the widespread implementation of these vaccines may face challenges due to the high cost of creating personalized vaccines for each patient. On the other hand, recombinant viral vector-based vaccines have shown the ability to enhance EBV-specific CD8+ and CD4+ T-cell responses in patients with NPC. Nevertheless, the safety aspect, particularly the potential risk of viral gene integration into the host genome, requires further evaluation. Therefore, developing new vaccines that offer increased immunogenicity and safety levels is essential.

2. Methods

2.1. Material

Set up two Petri dishes of RPMI-1640 and DMEM to form the control group. At the same time, add 10% FBS, 1% penicillin and 1% streptomycin to the two culture dishes. Put it into the cell incubator to maintain the temperature at 37 degrees and control the carbon dioxide to 5% [6].

Vaccine liposome LPS uses thin-film dispersion. Firstly, add equiproportional lipid substance and cholesterol molar solution and 2ml of dichloromethane into a distillation flask, distilled by a rotary evaporator, and then perform hydration and homogenization. Finally, uniform LPs are obtained.

Based on the amino acid sequence provided by the National Center for Biotechnology, mLMP2 was synthesized from the linearized DNA template by in vitro transcription. Pack mRNA into LPs at a 3:1 N/P ratio.

To assess the status of LPs and LPX, the average particle size and zeta potential of LPs were measured using a Malvern laser force analyzer, and all samples were tested two or three times below 25 degrees. Observing the appearance through the perspective of e-sports. Store LPX-mLMPS at 4 degrees to improve stability, and detect leakage and infection efficiency.

Mix a part of LPX-mLMP2 with 20% serum at a volume of 1:1, then let it stand at 37 degrees for 2 hours, and then put it into a centrifuge to control it at 4 degrees. The obtained liquid was removed from the supernatant, and mixed with normal saline, and LPX-mLMPS was dispersed in normal saline as a control.

Another aliquot of LPX-mLMPS was also mixed with serum and incubated at 2, 5, 10, 30 minutes and 1, 2 hours, respectively. The stability of each experimental group was compared.

2.2. Measurement

Assay of uptake cells using mCy5. Dendritic cells from commercial mice were seeded on gel well plates, cultured for 24 hours, and allowed to transfect LPX-mGFP. Finally, flow cytometry was used to collect and study the images.

TC-1 method was used to measure and verify antigen expression. TC-1 cells were seeded on 6-well plates, and cells were collected 24 hours later and washed twice with PBS.

2.3. Experiment

Dilute LPX-DiD with normal saline and inject it into mice. Six hours later, the mice were sacrificed and the individual organs were isolated. Then the IVIS spectrum living image system was used to detect the LPX expression.

Randomly divide the tumor-mouse into four groups. Comparison of saline, mLMP2, LPX-mir, or LPX-mLMPS treatment effects. (Mice were sacrificed five days later and individual organs dissected).

DOI: 10.54254/2753-8818/21/20230820

3. Results

3.1. Storage and Serum Stability of LPX.

The particle sizes and electrokinetic potential in colloidal dispersions, a measure of effective electric charge in nanoparticles [6, 7], of LPX-mLMP2 were shown as remain unchanged in the results. With the use of electrostatic interactions investigating serum proteins, the particle size of LPX-mLMP2 is shown as increased while the zeta potential decreased. On the other hand, an electropherogram is used in the investigation of the stability of LPX-mLMP2. This technique is to separate protein molecules based on their size and charge by applying current through a medium [8]. It is shown that LPX helped in the mRNA serum stability with incubation lasting for 2 minutes, 5 minutes and an hour. In terms of the transfection activity, as shown in the result, it has a 10% drop overall, due to the degradation of mRNA and the serum protein absorption, leading to a decrease in uptake inside cells. To conclude, LPs show a mRNA protective effect in vivo.

3.2. LPX Uptake in Cells

Using mCy5, the absorption of LPX by cells was investigated. The amount of LPX-mCy5 that was taken in increased and peaked at 4 hours. From the mechanistic analysis, caveolin-mediated endocytosis, a clathrin-mediated endocytic process involving the caveolae (bulb-shaped plasma membrane with a length of 50-60 nm) [9] and clathrin-mediated endocytosis, as shown in figure 1, the most common endocytic event in mammals [10] which plays a significant role in vesicular transport [9], were implicated in the cellular uptake of LPX-mCy5, since filipin and chloropromazine could considerably impede this process at the significance level at which the p-values equal to 0.05 and 0.001.

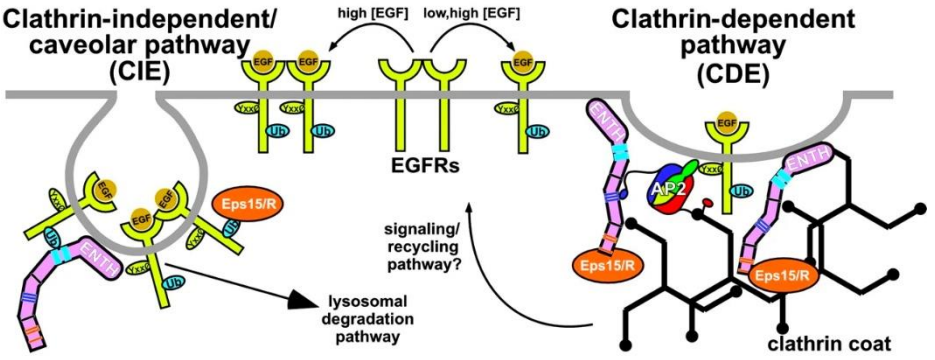

Figure 1. Diagram showing the different endocytosis mentioned in the text above [11]

3.3. Displaying and Presenting the Antigens

The transfection rates of blank LPs and mLMP2 were compared to those of the control. LPX-mLMP2 and Lipo-mLMP2 demonstrated transfection efficiencies that were greater than those of naked mLMP2According to this study, it was concluded that cationic carriers could enhance mLMP2 expression. However, lipo is more toxic than LPs and cannot be used in vivo. As naked mRNA is susceptible to destruction in the presence of nucleases, it is found that the production of the antigen is a critical stage in the immunization process.

3.4. Biodistribution and the in Vivo Translation of LPX.

It was prepared by injecting LPX- mLMP2/DID intravenously, through the vein, into mice to evaluate its biodistribution. The tissues were separated and examined after 6 hours. LPX-mLMP2/DiD was collected in some organs, including the liver, spleen and lungs. This could be because the nanoparticles' passive targeting abilities made them simply targets for phagocytosis, in which cells and particles are ingested and engulfed by phagocytes [12], by the mononuclear macrophage system. By using bioluminescence imaging, LPX-mLUC was used to examine translation efficiency in vivo.

DOI: 10.54254/2753-8818/21/20230820

Bioluminescence imaging has been developed over the last decade for molecular imaging of small laboratory animals, enabling the study of biological processes that are ongoing in long-lived animals [13]. This is comparable to the cationic lipids, which are the most commonly used for the transfection of genes, also known to have less mLUC expression in the liver than in the lung and spleen [14], DOTMA and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). The spleen is crucial for immunological control as it has the largest number of DCs. Therefore, DCs expressed in spleens were conducive to provoking an adaptive immune response in vivo.

4. Discussion

The blood samples were taken five days after administering the third immunization to mice with LPX-mLMP2. liver function test biochemical evaluations. It was concluded from the study that LPX-LMP2 had little hepatotoxicity and nephrotoxicity since all of the groups had values that were similar to those of the control group.

The tissues were separated, weighed, and applied with the hematoxylin and eosin staining method after immunization. Organ indexes showed no significant alterations, showing that the organs had not suffered harm as a result of the immunization. Also, the findings of the H&E staining revealed that the primary organs did not exhibit any evident histopathologic changes. LPX-mLMP2 was shown to be secure in vivo by these studies [6].

However, the cost of this technology is too high as this is a kind of biotechnology, and it always has a high cost in terms of the expensive operating costs in the biotechnology companies, including research, development, and testing that takes years to complete [14].

5. Conclusion

The cationic liposomes with mLMP2 encapsulation may productively express and display LMP2 antigen in vivo. After immunization, the tumor location shows an increase in antigen-specific T lymphocytes, the principal defense against NPC. Therefore, mRNA vaccination is an effective virus-related cancer immunotherapy. However, as the technology is still developing, it may not be generalized in the community within a short period of time.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230821

Effect of brain-computer interface training on functional recovery after stroke

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Abstract. As the aging population continues to grow, the incidence of stroke is increasing yearly. Patients with stroke often have residual motor dysfunction symptoms, which seriously affects their life and work. The traditional treatment methods have limited applicability and efficacy, making it difficult for patients to control their muscles voluntarily and achieve cortical-muscle coupling. Brain-computer interface (BCI) technology can enable patients with severely impaired motor function to control external devices through brain-controlled movements, thus promoting the efficiency of motor rehabilitation training and becoming a hot research topic. This article systematically summarizes the basic technology of BCI, its application in stroke rehabilitation, and future development trends.

Keywords: brain-computer interface, stroke rehabilitation, EEG decoding.

1. Introduction

Stroke has become the leading cause of death and disability in adults [1]. Motor dysfunction caused by stroke is the most common in clinical practice. 40%-60% of stroke patients will have varying degrees of residual symptoms, which can lead to a severe decline in their ability to live and work. In addition, nearly one-third of stroke patients also experience emotional disorders and mood swings [2, 3].

Currently, the clinical treatment of stroke mainly focuses on peripheral therapy for patients, with a lack of direct intervention in the patient's brain tissue, which has a significant impact on the patient's brain tissue and is often accompanied by long treatment times and poor efficacy [2, 4, 5]. Therefore, there is an urgent need to adopt more effective and safe intervention measures for stroke patients to improve their survival status and enhance their quality of life.

Researchers have found that brain-computer interface (BCI) technology can effectively treat patients with motor deficits in clinical practice [6]. There are two types of BCI modes used for the diagnosis and treatment of limb movement disorders. One is an assistive BCI, which uses BCI devices to decode the patient's intention to move, thereby controlling external devices such as robots. Unlike traditional prostheses that only have slots for the residual limb, this BCI-based prosthesis can be directly connected to the residual limb's nerves and muscles, allowing users to control it using their brains and achieving a sense of reality during use. The other is a rehabilitation BCI, which can directly intervene in the patient's brain. Because the human nervous system is plastic, repetitive feedback to the

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DOI: 10.54254/2753-8818/21/20230821

brain through BCI devices can strengthen the connections between neurons and achieve the goal of treatment [6, 7].

1.1. Signal acquisition

According to the signal acquisition method, BCI technology can be divided into invasive and non-invasive types. Invasive BCIs mainly use ECoG and Spikes for signal acquisition. In contrast, EEG is the primary method for non-invasive BCIs. The difference between these two signal acquisition methods is that ECoG and Spikes require the implantation of electrodes into the patient's brain cortex, while non-invasive BCI records brain signals non-invasively from the patient's scalp. Because invasive BCI signal acquisition methods have a higher spatial resolution, better signal-to-noise ratio, and wider frequency bands, but ECoG and Spikes require the implantation of electrodes into the user's brain cortex, which can lead to rejection reactions, deterioration of signal quality, and degradation of electrode performance over time. However, EEG has higher safety, more convenient acquisition, and lower cost because it does not require the implantation of foreign bodies through surgery.

1.2. Signal processing and decoding

The brain signals can be transformed from patterns of neural activity to neural activity data. However, the brain signals contain noise signals that affect signal quality, such as neural signals unrelated to patient psychological activity, power frequency interference, electro-ocular, and muscle artifacts. These noise signals may be caused by motion, such as blinking, eye movement, and head movement. Different preprocessing methods are applied to filter out noise unrelated to the user's psychological activity. However, in the process of filtering noise, maintaining the signal’s integrity is also a difficult problem.

After preprocessing, the brain signals will extract features based on the neural signal rules of different BCI modes, and then train the classification model using pattern recognition technology or machine learning algorithms. However, there are still many unsolved but essential problems in the decoding aspect of current BCI technology:

1. The information output rate of current BCI technology is limited, seriously affecting external devices' functionality.
2. Data fidelity is also vital for functional application BCI.
3. Due to individual user differences, most current classification models must be customized for specific users.

The customization process requires much time to train the model using the user's brain signals. An efficient and suitable model can only be found through a large number of model training.

1.3. Device control

Many external devices can be communicated with and controlled through BCI. In the case of communication or control applications, the control interface can translate the decoded logical control signals represented by the user into semantic control signals and then convert these into physical control signals [4, 6, 8]. BCI technology can be used in stroke rehabilitation to convert brain information into conscious movements of disabled limbs. In addition, existing BCI technology can allow users to trigger upper limb exoskeletons through motor imagery-based BCI devices, assisting patients with grasping, releasing, and arm-body movements. BCI can also be used for lower limb movement function rehabilitation, assisting the body in completing supporting walking and other movement functions [7].

2. Application of brain-computer interface in stroke rehabilitation

The main goal of stroke rehabilitation is the restoration of motor function. However, due to the limited effectiveness of traditional treatment methods, there is an urgent need for more effective treatment options. The emergence of BCI has become the most promising tool for motor function recovery in the future.

DOI: 10.54254/2753-8818/21/20230821

2.1. Application of BCI in stroke treatment

Motor function recovery is the most researched application of BCI stroke. BCIs mainly help patients with impaired motor function after a stroke to improve their quality of life through two methods. The first is an assistive BCI, which completely bypasses the damaged neural pathway and controls external devices such as prosthetics or exoskeletons through the BCI device, receiving the patient's motor intention. For example, an intelligent prosthetic limb system consisting of a neural-muscle-bone prosthetic limb, a mechanical arm and hand controlled by the motor cortex signals of the brain, and a hemiplegic stepper and interactive gait correction device.

The second method is a rehabilitative BCI, which effectively promotes neuroplasticity to restore damaged neural connections and thus restore impaired functions. For example, the BCI upper limb motor recovery system controlled by motor imagery and the BCI synchronous closed-loop rehabilitation system combined with virtual reality technology [9-11].

2.2. Advantages of BCI rehabilitation technology

Compared to traditional rehabilitation methods, BCI can be applied to a broader range of patients with post-stroke motor impairments. Before the widespread understanding of BCIs, traditional post-stroke motor impairment treatments mainly included occupational and constrained-induced movement therapy. However, patients with severely impaired motor function do not have the minimum required motor ability for conventional rehabilitation programs [6, 12]. In contrast to the conventional approach, BCI relies on the user's brain signals to drive the interface. Therefore, even stroke patients who have completely lost their motor control ability can imagine movement and activate the cortex associated with motor actions. Studies have shown that even imagining a motor action can produce brain activation consistent with actual movement. In addition, BCI can help patients establish a connection between their brain's intention and external assistive devices, inducing patients to achieve self-regulation and coupling coordination between the cortex and peripheral neuromuscular systems under the plasticity rehabilitation mechanism.

2.3. Adverse reactions and limitations of BCI

Based on the above content, BCI has received extensive attention and development due to its wide range of applications and significant therapeutic effects. However, as an emerging, complex, and interdisciplinary communication technology, many problems still need to be solved. First, the information acquisition technology of BCI still needs to be improved. Non-invasive BCI detects weak signals, but if electrodes are implanted in the brain, the implanted electrodes are wrapped by inflammatory cells over time, which can lead to signal loss. In addition, BCIs also need to address security risks such as hacking attacks, mind control, and data theft, which can lead to privacy leaks [11].

3. Future outlook

With the significant improvement of medical technology, BCI technology has been widely recognized and developed rapidly, showing good research and application prospects in post-stroke motor recovery. However, BCIs are still in the early development and theoretical exploration stage in this field. If we want to apply this technology widely to clinical practice, we still need to face many challenges.

The first challenge is to solve the problem of bidirectional interaction in BCI technology. Although BCI technology has made significant progress in motor recovery, the recovery of a limb requires both motor function and sensory ability (such as touch and proprioception) to be restored. By combining kinetic and sensory functions, bidirectional BCI and closed-loop systems can be merged to adjust the user's movement pattern based on sensory feedback. Studies on neural prostheses based on bidirectional brain-machine interfaces have been conducted, and this problem is believed to be resolved soon [13-15].

The second challenge is the application of synchronous and asynchronous BCI. The BCI systems for post-stroke recovery mentioned above, such as mechanical arms, mechanical hands, and paraplegic

gait trainers, all use synchronous BCIs. The working principle of synchronous BCI systems is to obtain the user's real-time brain signals, which also means that patients need to continuously "work" to produce brain signals to control the BCI system. However, users are idle most of the time when using BCI external devices. Only BCI that can correctly identify the user's idle state and generate idle-state actions through asynchronous BCI can avoid system errors and improve the availability of online systems and user autonomy [13, 16].

Finally, the research direction of hybrid BCI technology, which uses electroencephalography and other physiological signals such as electromyography and heart rate, is also a challenge. These combinations can work together to control algorithms more accurately and improve the reliability of detecting user intent results [17].

4. Conclusion

In recent years, there has been significant research progress in hardware and algorithms in the field of brain-computer interface technology. As an interdisciplinary field, BCI has extensive research and application prospects in the medical field, and this technology has already achieved exciting results in stroke rehabilitation. We look forward to the future development of this critical research field and hope that this technology can benefit everyone.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230826

Albumin-binding prodrugs' use in treating cancer

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Abstract. Throughout the past few decades, the use of targeted delivery methods for cancer therapies has dramatically increased. Prodrug, a type of drug that can apply the targeting therapy, is defined as chemicals that undergo chemical transformations after enters the patient's body. They will be activated and release the parent drug after reached the targeted site. Both passively and actively targeted therapies are explored. The albumin-binding prodrug, a model of prodrug applying passively targeted therapy, this paper introduces the enhanced permeability and retention (EPR) effect to target the tumor site. Besides, since the human serum albumin is an endogenous substance originated from human body, its nanoparticles is able to carry the anticancer drugs to elongate the circulatory half-lives of drugs since it will not be rejected by the immune system. However, the activated compound's systemic toxicity and the dearth of information regarding the biodistribution of prodrugs are two possible disadvantages. Moreover, some exogenous albumin formulations are prohibited from participating in clinical trials because of their very poor delivery efficiency. In conclusion, the albumin-binding prodrug is a cancer treating therapy that has great promise and great capability despite the potential risks.

Keywords: albumin-binding prodrugs, anticancer, the EPR effect.

1. Introduction

Recent decades, scientists have explored extensively in the targeted drug delivery system. Now, they can deliver the anticancer drugs to the tumor tissue efficiently [1]. The nanomaterials such as polymeric nanoparticles, dendrimers, micelles, liposomes and other inorganic nanoparticles are especially focused [2]. The prodrug, which is defined as a chemical that is transformed before it has pharmacological effects, are used in the targeted therapy since the side-effects of the toxic drugs can be reduced when the number of drugs is reduced.

The passively targeted therapy is developed with the help of the EPR effect. The nanoparticles are able to penetrate the cleavage between the endothelia cells in tumor tissue and finally accumulate in them, due to the enhanced permeability and retention. There are also some approaches to deliver the activating enzymes, which are considered actively targeted therapy. For example, by using the chemical trigger, the therapy may let the enzyme be a conjugate to an antibody, a polymer-based nanoparticle, a virus or even an entire cell [3]. In addition, the physical trigger can be used to activate the prodrug from the exterior. The photodynamic therapy (PDT) is now extensively explored and has become an effective treatment to many types of solid tumors. By using this therapy, the unreactive prodrug will be activated by visible lights or near-infrared light and then be converted to toxic drug [3]. Advantages of using PDT includes less harmful radiation. Also, PDT will not weaken the immune system like what the

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DOI: 10.54254/2753-8818/21/20230826

radiotherapy does, since it will not decrease the amount of white blood cells (WBCs) and other immune system cells.

However, there are some shortcomings in this system, such as the potential toxicity of the nanocarriers and the complex structures which has limited the mass-production of the drugs. Also, only a few drug candidates are allowed to be used in clinical trials.

This paper explains what prodrugs are and focuses on an example of albumin-binding prodrug called Aldoxorubicin. Besides, this paper gives accounts of the hydrazone linker, doxorubicin drug, the EPR effect applied, and the positives and negatives of prodrugs.

2. Prodrugs

When body cells are growing and spreading in a human's body uncontrollably, this person is considered getting cancer. Cancer cells are multiplying and invading abnormally, they sometimes form malignant tumors, which can continuously spread to adjacent tissue. The energy and nutrients the tumor cells needed is increasing dramatically at time passed. One difficulty of treating cancer is the poor tumor site selectivity [4]. One direction that scientists are exploring is to improve the targeting ability of drugs, which means the drug should precisely attacking the cancer cells instead of the normal cells.

Prodrug is drug molecules that undergo enzymatic and chemical transformations to release the active parent drug [5]. Prodrugs are designed in two ways, one is called bioprecursor, another one is called carrier-linked prodrug. The bioprecursor is a simple compound that transforms into an active parent drug metabolically or chemically [6]. The carrier-linked prodrug is an active molecule linked to a carrier through a covalent linkage. It will release parent drug and the carrier to become active drug after metabolic hydrolysis [7]. The carrier-linked prodrug is pH-sensitive, which means it utilize the unusual acidic environment caused by cancer to cleavage hydrazone linkage.

3. The construction of an albumin-binding prodrug aldoxorubicin

The construction of the albumin-binding prodrug includes a drug, linkers, and a peptide. The specific prodrug this paper introduced as an example is called Aldoxorubicin, a prodrug of doxorubicin (DOX). In this prodrug, the linker to albumin is maleimide moiety, the linker to drug is hydrazone, and the prodrug binds with human serum albumin [8]. The maleimide moiety of the prodrug reacts rapidly and selectively with the cysteine-34 position of endogenous human serum albumin (HSA) via Michael addition. After that, DOX is released from the albumin carrier by the cleavage of hydrazone bond in the acidic environment of cancer cells. The Aldoxorubicin has been investigated in clinical trials. It can improve the pharmacokinetics and biodistribution of mice with tumor and can effectively bind with endogenous albumin. Comparing with free DOX, the prodrug Aldoxorubicin has greater targeting ability and better therapeutic effect.

3.1. Hydrazone

The reason why hydrazone is chosen to be the linker of drug is due to its chemical property. The hydrazone relates to ketones and aldehydes, it releases free drug through hydrolysis once an ADC is transported to acidic endosomes (pH 5.0–6.0) and lysosomes (pH about 4.8).

At the same time, cancer cell will always create acidic environment. The rapid growth of cancer cells requires a lot of oxygen and energy, which leads to three properties: hypoxia, glycolytic tumor cell metabolism, and inefficient blood perfusion.

Another mechanism for cancer cells to create acid is the Warburg effect. In normal cells, energy was produced through the usual citric acid cycle and oxidative phosphorylation in the mitochondria. However, the Warburg effect explained that most cancer cells go through a less efficient process of "aerobic glycolysis" consisting of high level of glucose uptake and glycolysis followed by lactic acid fermentation taking place in the cytosol, even in the presence of abundant oxygen [9]. To increase the rate of glucose uptake, lactate join the final electron transfer chain in the aerobic glycolysis. So, the byproduct is lactic acid and lactate.

DOI: 10.54254/2753-8818/21/20230826

Hydrazone linkage is pH-sensitive, it will only break in acidic environment, which is formed by the lactic acid produced by tumor cells. Thus, the hydrazone linkage will only release the drug at where the cancer cell exists, which represents the targeting ability of the hydrazone linkage.

3.2. Human serum albumin

HSA, also known as human serum albumin, is an essential nutrition supply for the body. Due to its numerous benefits, such as good biodegradability, easy surface modification, and high biocompatibility, albumin has been intensively researched as a natural medication delivery mechanism [2].

The target site of cancer cell. It is a global plasma protein, and the structure is shown in Figure 1.

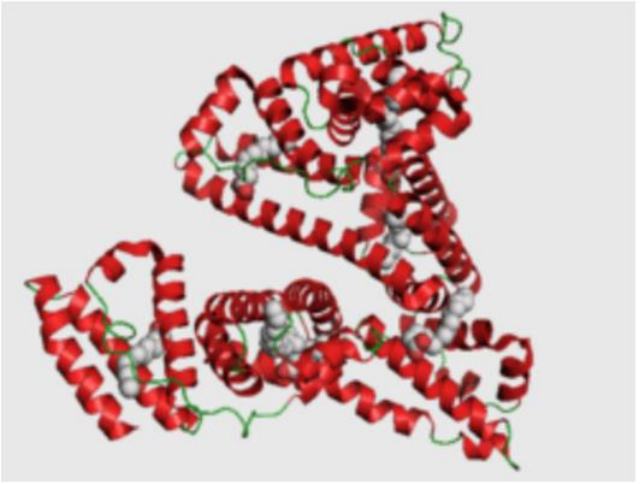

Figure 1. The structure of human serum albumin [10].

Through the protein nanoparticle generation, HSA as the carrier will pack the drug inside and enter the cell by endocytosis. After getting into the tumor cell, the HSA nanoparticles and drugs will accumulate.

One of the reasons HSA is selected is because it is an endogenous substance, which means it can be produced by human body itself. Thus, the prodrug carried in the HSA will not be recognized as enemy by the immune system. So, the advantages of using HSA have included increased stability and activity, decreased enzymatic degradation, decreased immunogenicity, decreased phagocytosis, and decreased renal clearance.

4. The EPR effect

EPR effect, also known as enhanced permeability and retention effect, is an important mechanism that plays an important role when the HSA nanoparticles are functioning [10]. In normal tissue, there are densely connected vascular endothelium and healthy lymphatic system. The closely packed endothelial cells prevent the nanoparticles from crossing the blood vessel, and the lymphatic system allows body fluid returns to the circulatory system.

However, in tumor tissue, there are hyper-vascular, abnormally organized leaky vasculature, and ineffective lymphatic system. There will be leakages between the endothelial cells which allow nanoparticles with diameter 100-800nm to pass through, and that's what the permeability on the name of the EPR effect means. When tumor has a volume that is larger than 2 mm3, its diffusion will be limited [11]. This limitation will affect nutrition intake, waste excretion, and oxygen delivery. To overcome this limitation, the tumor will increase the surrounding vasculature which is called angiogenesis, and thus get more excess to the energy, nutrients and oxygen. The tumor cells undergo angiogenesis have basement membrane that are abnormal and has distorted shape and do not have normal amount of pericytes lining endothelial cells. In different tumor types, the gap may have a size of a range from 100 nm to 2 μm.

DOI: 10.54254/2753-8818/21/20230826

The retention effect is due to the ineffective lymphatic system. The tumor cells do not have well-developed lymphatic system. So, there will be a high interstitial pressure which will force out the fluid and causing a convective interstitial fluid flow towards the periphery of the tumor, which prevents the accumulation of drugs toward the center of the tumor. However, the nanoparticles will have longer retention time due to the leaky vasculature and poor lymphatic drainage. They limited the nanoparticles to return to the tissue fluid (Figure 2). Finally, the nanoparticles, including the prodrugs, which are smaller than the leakage will reach the interstitium and be packed in the tumors [12].

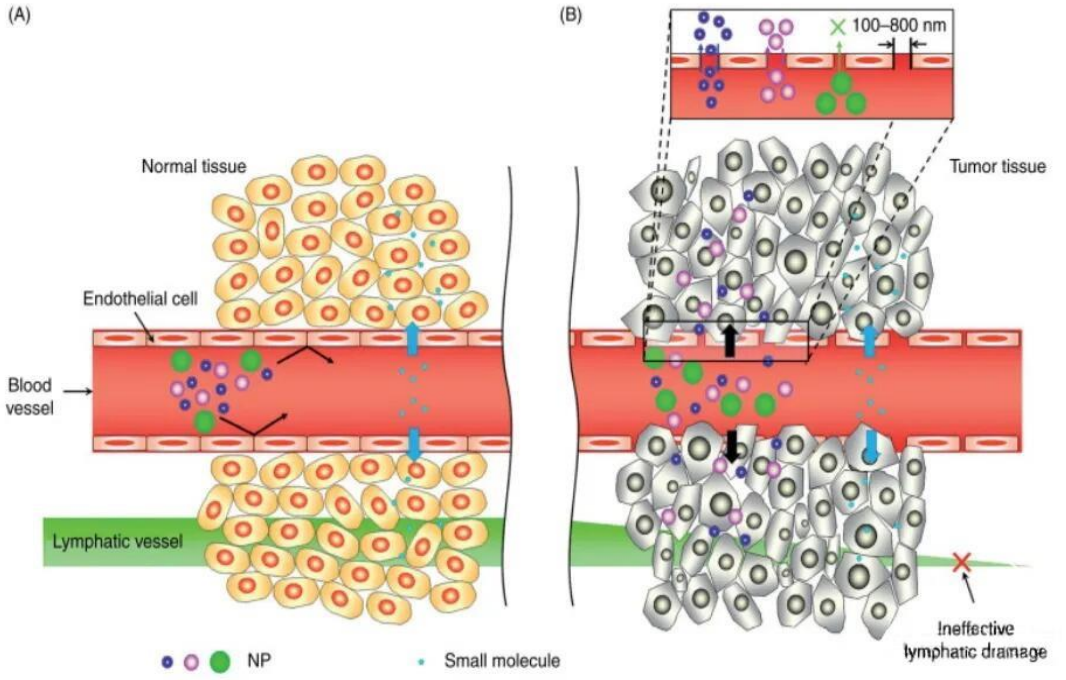

Figure 2. (A) The comparison between the condition of normal tissues; (B) the condition of tumor tissues [13].

The EPR effect is the strategy that the researchers have explored the most [1]. However, although some tumor cells are in high-EPR xenografted tumors, the nanoparticles can only accumulate a small percentage, even less than 1%. One reason may be the physical barriers such as the endothelial barriers, cellular barriers, or the Kupffer cell. Another reason may be the significant stochasticity in the extravasation of NCs across the tumor vascular.

5. The receptors of HSA

After the drugs pass through the endothelium cells, they are able to bind with special receptors over-expressed on the tumor cells, going through a pathway of GP60 receptor, cavoline-1, and SPARC receptor. Some of them can increase the half-life of the albumin.

The glycoprotein (GP) 60 receptor, also known as albondin, is an albumin that exists on the membrane of the vascular continuous endothelia cells and alveolar epithelial cells. It can allow the biodegradable albumin be transported and recycled without being degraded by lysosome [2, 12]. The GP60 can bind with the HSA nanoparticles and form caveolae called cavoline-1, which is an albumin-containing vesicle. The cavoline-1 enters the cytoplasm, fuse with the basement membrane, and thus transport the albumin to the interstitium [14].

DOI: 10.54254/2753-8818/21/20230826

6. Positives and negatives of prodrugs

6.1. Positives

About 10% of drugs in the world is prodrug. The prodrugs have two main benefits: increased effectiveness and decreased side-effects. It can help medications travel to the target site (where they need to work), and impact how medications are distributed at the specific site [15].

6.2. Negatives

At the same time, there are some potential drawbacks such as systemic toxicity of the activated compound and the lack of data about the biodistribution of prodrugs. In addition, some exogeneous albumin formulation are not allowed to do clinical trials due to its unexpected low efficiency of delivery.

7. Conclusion

This paper discussed the albumin-binding prodrugs' use in treating cancer, including the explanation of prodrug and an example called Aldoxorubicin. The construction and reaction mechanism of Aldoxorubicin has been discussed respectively, such as the hydrazone and the human serum albumin. To sum up, although only a few candidates are used in clinical trials, the albumin-binding prodrug is still a promising cancer treatment due to its targeting ability. In the future, scientists may not only focus on the toxicity that needs future experiments to reduce, but also make more efforts on exploring the ways to improve the targeting ability of the prodrugs.

To improve this paper, more detailed information about the use of doxorubicin and maleimide moiety may be added. Also, there could be more discussion and more data on the positives and negatives of prodrug by referring larger variety of papers.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230827

Recent progress in the pathology, diagnosis, and treatment of Alzheimer's disease

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Abstract. Alzheimer's Disease (AD) is one of the prime cuases of dementia, responsible for 60% to 70% of cases worldwide, according to the World Health Organization (WHO). Unfortunately, numerous research challenges still remain for this disease, which poses a great threat to human health worldwide, especially in the elderly population. Scientists are still struggling to find the pathogenesis and pathogenic mechanisms of AD, and while research for new tests and novel drugs is ongoing, it faces a high failure rate. This article will summarize some remarkable results to date and discuss future research directions.

Keywords: PET scanning, Alzheimer's Disease, Amyloid-beta (Aβ), Synaptic Dysfunction

1. Introduction

AD is a progressive neurodegenerative disease that located in the central nervous system and primarily occurs in presenile and elderly populations. The amount of people who suffer from AD cases among the whole world is continuously rising, especially among the elderly group. According to a report published by the Alzheimer's Association in 2015 the rate of getting AD among the elderly population in the United States will increase from 11% to 16% by 2050 [1]. This rising trend is developing in the aging population worldwide, not only in the United States. AD can cause several complications, including but not limited to depressed mood, memory loss, decreased ability to live, and ultimately, death, at the end. Based on the report of Alzheimer's Association in 2022, 33.3% of the elderly died from AD or other dementia, and AD ranked at the seventh leading cause of death in the US during 2021-2022 [2].

As a disease with slow and insidious onset, the pathophysiological process of AD may begin much earlier the formal diagnosis of dementia [3]. The long development process of AD can be summarized as follows: the preclinical stage, mild cognitive impairment (MCI), and dementia. The clinical manifestations of AD include aphasia, apraxia, agnosia, cognitive decline, mental disorder symptoms, behavioral disturbances, declination in living ability, and memory impairment. The level of deterioration in cognitive and physical functions manifests in different symptoms at different stages. The etiology, diagnostic criteria, and treatment methods of AD are complex puzzles that have not yet been completely resolved. Therefore, developing a more comprehensive understanding of the etiology, pathogenesis, and biomarkers of AD through research, detecting the possibility of AD as early as possible or even in advance, developing tactics for AD prevention in the preclinical stage, and treatment methods with a substantial curative ratio is essential to reduce the prevalence, mortality, and medical burden of AD.

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DOI: 10.54254/2753-8818/21/20230827

2. Pathology

2.1. Amyloid-beta accumulation

Amyloid-beta (Aβ) production can be broadly summarized as follows: β-secretases cut amyloid precursor protein (APP) at β-sites Asp1 and Glu11 to produce C-terminal membranes with 89 or 99 amino acid fragments attached (C89, C99). BACE1 is the predominant β-secretase; however, recent studies suggest that BACE2 and cathepsin B should also be considered as additional β-secretases [4,5]. C89 and C99 were subsequently cut by γ-secretases, a multi-subunit protease complex assembles by presenilin1 (PS1), presenilin 2 (PS2), anterior pharynx defective 1 (APH-1), presenilin enhancer 2 (PEN-2) and nicastrin (NCT), in the transmembrane domain, and cleaved to produce Aβ1-40/1-42 and Aβ11-40/11-42 [6-11]. It was found that mutations in APP, progerin, apolipoprotein E (APOE4), and abnormal levels of neuropeptides may contribute to plaque formation of Aβ [12,13]. Aβ accumulation may contribute to impaired synaptic plasticity and neuronal cell death, resulting in tau hyperphosphorylation, excitotoxicity, and the generation of ROS. Thus, Aβ acts as a critical component in AD pathology and has a great potential to become a major direction for research on AD etiology as well as targeted drugs.

2.2. Neuroinflammation

Aβ precipitation and neuroinflammation are closely interrelated. Aβ precipitates activate Toll like receptors (TLRs) and immune cells, such as microglia and astrocytes. Prolonged activation if these cells produce pro-inflammatory cytokines of the IL-1β family, which increases inflammation. Tiina M Kauppinen et al. injected Aβ peptide into the brain of hAPP(J20) mice and observed the effect of PARP-1 on Aβ-induced microglia activation. Their results demonstrate that Aβ can induce microglia activation by an unknown mechanism, and that PARP-1 inhibition is able to block Aβ-induced microglia responses without impairing microglia phagocytosis of Aβ peptide [14]. Jill A White et al. treated rat astrocytes with oligomeric Aβ and found that it induces high levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), iNOS, nitric oxide (NO) and TNF-α, leading to a significant inflammatory response [15]. Aβ also induces neuroinflammation and enhances NO synthesis, which makes Aβ plaques more difficult to be cleared by microglia, and even increases Aβ plaque formation, and inhibits synaptic plasticity [16]. Thus, Aβ induces neuroinflammation and other negative effects, and the generation of pro-inflammatory cytokines (occur in neuroinflammation), in turn, leads to increased Aβ plaques, which created a vicious cycle.

2.3. Mitochondrial dysfunction

Mitochondrial dysfunction is caused by impaired mitochondrial cytoarchitecture as well as altered electrochemical gradients. Ongoing fission and fusion events impact the size and number of mitochondria. Both mitochondrial fission and fusion proteins (including DRP1, Mfn2, OPA1, etc.) are vital elements during these processes [17]. Researches have shown that the expression of almost all mitochondrial fission and fusion proteins ia altered in the brains of patients with AD [18-21]. Although the reasons behind these changes and how they lead to structural mitochondrial damage are not clear, it is notable that the reduced number of mitochondria in biopsied brains is accompanied by a significant decline in mitochondria length but an increase in width, leading to an overall swollen apprearance of mitochondria.

Mitochondrial dysfunction leads to a decrease in ATP production efficiency but an increase in reactive oxygen species (ROS) production efficiency, a high-energy phosphate compound that provides energy supply for cellular life activities. ROS are chemically reactive oxygen-containing chemicals, and an increase in ROS can cause oxidative stress and severe cellular structure damage. And the decrease in ATP and increase in ROS due to mitochondrial dysfunction may be the main cause of the oxidative imbalance in AD [22,23]. In turn, research has shown that mitochondrial dysfunction is a significant sign for the early stages of AD [24]. Mitochondrial dysfunction may also lead to other adverse effects,

DOI: 10.54254/2753-8818/21/20230827

such as calcium overload and dysregulation of calcium homeostasis in the endoplasmic reticulum (ER) [25].

2.4. Synaptic Dysfunction

Synaptic Dysfunction shows a strong correlation with cognitive decline in AD patients. It is a more obvious indicator of cognitive deficits than neurological death [26]. Synaptic Dysfunction is associated with oxidative stress, ROS, Aβ, hyperphosphorylated tau protein, loss of mitochondrial function, and altered metal homeostasis, forming a complex cycle [27].

Soluble Aβ peptide oligomers prompt synaptic loss. Aβ triggers cytoplasmic $Ca2^{+}$ elevation, abnormal redox and downstream pathways activation [28-30]. Oligomeric Aβ disrupts glutamate uptake and activates astrocyte glutamate to increase extrasynaptic domain glutamate levels. This leads to excessive activation of eNMDARs and mediates excitotoxicity, producing excess amount of reactive nitrogen species (RNS) and ROS. Furthermore, excess RNS and ROS lead to abnormal oxidative reactions in proteins, resulting in synaptic damage [31-37].

Tau protein may be also involved in the development of synaptic dysfunction. Tau is a type of microtubule-associated protein (MAP) which occurred mainly in CNS neurons' axons [38]. Its main function is to act together with other MAPs to help and promote microtubule (MT) assembly [39,40]. Under pathological conditions of tauopathies, including AD, tau protein can hyperphosphorylated, detach from microtubules, and form soluble aggregates and insoluble filaments in specific areas of the brain, eventually forming neurofibrillary tangles (NFTs) [41,42]. Compelling evidence demonstrates that excessive activation of extra-synaptic NMDARs by oligomeric Aβ leads to increased p-tau levels, indicating Aβ induced tau hyperphosphorylation. Hyperphosphorylated tau interacts abnormally with the mitochondrial cleavage protein Drp1 in brain autopsies from AD patients, revealing that tau may actively involve in Aβ-induced mitochondrial dysfunction. However, further research is required to discover the exact mechanism [43,44]. Additionally, Jadhav et al. indicated that presynaptic tau protein is associated with synaptic loss and synaptic failure and that low levels of presynaptic protein may be relevant with a growing number of NFTs [45]. Other studies have also demonstrated that tau fragments can diffuse across synapses and affect postsynaptic neurons, leading to neurogenic fiber degeneration with implications for synaptic function [46].

3. Diagnosis

3.1. PET

Positron Emission Computed Tomography (PET) is a relatively advanced imaging technique in modern clinical examinations. PET technology has also demonstrated unique advantages in the examination of patients with neurological and psychiatric disorders, and has a great future prospect for the diagnosis of AD, treatment planning, and assessment of disease progression in ongoing studies. [47]

PET works by labeling a substance, such as glucose, which is essential to the metabolism of biological life with short-lived radionuclides. These nuclides are capable of releasing positrons during decay and annihilate upon encountering one, resulting in a pair of photons with an energy of 511 KeV. A highly sensitive camera will capture this pair of photons and obtain a three-dimensional image of the aggregation of the labeled substance in the organism, reflecting its metabolic activity through calculation and analysis [48].

The use of Aβ deposition levels, tau aggregation, and neuroinflammation as PET tracers in the biomarker of AD is the dominant direction in current research, which targets the role PET imaging can play in AD detection.

3.1.1. 11C and 18F labeled Aβ tracers. In 2004, Klunk et al. found that 11C-labeled Pittsburgh compound B ([11C] PiB), a radioligand, was able to diagnose mild AD in patients by detecting higher levels of ligand residues in regions such as the frontal and temporoparietal cortices [49]. Current studies have shown that PiB PET may also be applicable to the diagnosis of mild cognitive impairment (MCI,

DOI: 10.54254/2753-8818/21/20230827

mid-AD) [50,51] . The increase in amyloid load is the basis of PiB PET. However, significant elevations in amyloid load are not universal in MCI patients. Therefore, 11C-labeled tests for amyloid deposition levels are not a strong indicator in the diagnosis of MCI [52, 53].

The development of three 18F-labeled Aβ tracers, [18F] florbetapir, [18F] florbetaben and [18F] flutemetamol, has improved the detection capability of PET scanning in MCI patients [47]. Compared to [11C] PiB, 18F-labeled tracers have demonstrated advantages in recent studies with longer half-life, higher sensitivity and specificity, higher brain kinetics, and higher cost-effectiveness [47,50,52,54,55].

The extent to which the results from PET measuring Aβ deposition are reliable and valid is controversial, and this assay has limitations [56]. This is because the association of Aβ load with patient cognitive awareness, hippocampal atrophy, brain atrophy, the rate and velocity of Aβ deposition, and the sensitivity to amyloid-positive thresholds varies between patients at different stages, which are all possible influencing factors and should be a future research direction to improve the benefits of PET measurements in AD diagnosis [57].

3.1.2. Generation development in tau tracers. The first-generation tau tracer, THK tracers (especially the newer versions [18F]THK5351 and [18F]THK5317), have shown exceptional capability to differentiate MCI patients from healthy patients, with particularly high retention in temporal brain regions, along with good pharmacokinetic profiles [58]. [18F]MK-6240, a second-generation tau tracer, exhibited high binding and affinity to tau deposits [47]. Another type of second-generation tau tracer called [18F]PI-2620 was able to demonstrate tau binding in more regions, including the parietal and temporal lobes, providing more possible foundation for diagnosis. It also shows high binding and affinity, and improes the lack of off-target binding which is the shortage of first-generation tau tracers [59, 60].

In contrast to PET assay studies for amyloid deposition, there has been little progress in the research of tau aggregates as a biomarker and its use in AD detection [47]. Because tau is located inside cells, the ligands used in the assay need to have the ability to cross multiple layers of barriers. Additionally, tau aggregates are less concentrated throughout the brain than Aβ deposits, so the ligands also need to possess greater specificity than Aβ tracers [47]. Based on the current research progress, these ligands show relatively good prospects in AD diagnosis.

3.1.3. TSPO tracers for neuroinflammation. Translocator protein 18 kDa (TSPO) is present in glial cells and ventricular membrane cells [47]. It has been shown that in the presence of neuroinflammation, microglia are activated, and TSPO levels are significantly upregulated [61]. This phenomenon demonstrates that the detection of activated microglia by TSPO can measure neuroinflammation [62].

The first-generation TSPO tracer, [11C]PK11195, has been found to show a decrease in microglia activation in the pre-AD period, but an increase in microglia activation in the subsequent disease progression [63, 64]. This phenomenon was later called the bimodal hypothesis. Although [11C]PK11195 is the most well-studied tracer, it also has technical limitations due to its very short half-life, low uptake, low signal-to-noise ratio, high non-specificity, and problems with suboptimal modeling [65]. The second-generation tracers had shown a remarkable improvement within the signal-to-noise ratio and binding affinity [66]. These studies suggest an explanation for the bimodal hypothesis of AD neuroinflammation by combining the results of several studies (the second-generation tracer [18F] DPA-714 and the first-generation tracer [11C] PK11195): the peak of activated microglia in early patients serve a protective purpose of clearing Aβ deposits, whereas the later peaks are deleterious [63]. However, the second-generation TSPO tracers also have the limitation that they exhibit high sensitivity to different binding affinity patterns due to single nucleotide polymorphisms (SNPs) in the TSPO gene. Testing for these genetic polymorphisms is unavoidable in the detection of neuroinflammation using TSPO [47].

3.2. MRI

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique to visualize the internal structure of human body without exposing the patient to radiation. It provides high resolution images of

DOI: 10.54254/2753-8818/21/20230827

body parts, especially soft tissues, by detecting and presenting the internal body structure by using energy with different attenuation released by nuclear magnetic resonance of nuclei [67].

The advantage of MRI testing in the clinical diagnosis of AD is its ability to measure neurodegenerative atrophy in different parts of the brain. Brain atrophy in AD patients starts in the medial temporal lobe (internal olfactory cortex and hippocampus), then spreads to the inferior temporal lobe and parietal limbic cortical regions, and finally to the multimodal joint neocortex, leading to the transformation from MCI to dementia [68]. MRI can detect changes in specific locations involved in brain atrophy, such as cortical thinning in the lateral and medial parietal, posterior cingulate and lateral temporal cortices, as well as volume atrophy in the hippocampus [69]. These changes are biomarkers of AD and can provide valuable evidence supporting the diagnosis of AD.

3.3. Fluid Diagnosis: CSF & Blood

Biomarkers, a crucial part of AD pathology, largely contribute to the clinical diagnosis of AD, especially in the examination of body fluids (CSF and blood).

3.3.1. CSF. Cerebrospinal fluid (CSF) testing is a diagnostic method which can help diagnose disease by examining changes in the composition of the specimen under pathological conditions. Aβ42 and Aβ42/Aβ40 ratio are important biomarkers in AD and prodromal AD, and their average level of change is about 50%. Their changes in these biomarkers reflect individual differences in brain amyloidosis and Aβ production or secretion, which are consistent with the results of Aβ tracers in PET. High T-tau and P-tau indicators are also pathological traits of dementia and prodromal AD, with an average change level of about 250%. However, T-tau values can change in many neurodegenerative diseases, depending on the severity of the neurodegeneration or lesion, wherase increases in P-tau can only occur in AD. Neurogranin is a promising biomarker that has emerged in recent studies and may be specific enough to meet the heterogeneity requirements of late-onset AD pathology. High neurogranin can reflect the degeneration of dendrites that characterizes the pathology of AD and prodromal AD [70].

3.3.2. Blood Testing. Blood tests involve drawing blood and analyzing the composition of the sample to detect lesions or other abnormalities in the body. The application of blood tests in AD's clinical diagnosis is a new breakthrough, and further studies are needed to fully understand their potential benefits and limitations, thus providing a stronger basis for the diagnosis of AD. Studies have shown that reduced Aβ42/Aβ40 ratio and high APP669-711/Aβ42 ratio are associated with positive brain amyloid and may be useful as a screening technique for the detecting brain amyloidosis. Increased plasma Tau is a biomarker for acute neuronal injury, including AD and some other neurological diseases; High plasma neurofilament light (NFL) is a pathological marker for AD and prodromal AD, but like tau, is not specific for AD [70]. More research is needed to optimize the use of blood tests in AD diagnosis and to identify additional biomarkers with greater specificity.

4. Treatment

As the most direct means to interfere with the progression of AD and potentially reducing its prevalence of AD at its source with preventive effects, the treatment of AD has been a topic of great interest and a major difficulty in research. The incomplete understanding of the pathology has led to stagnation in the development of targeted drugs. Due to its insidious onset and continuous progressive development, the pathological changes caused by AD may begin long before symptoms occurred, which makes the detection and treatment of the underlying pathology more difficult. The following sections summarize the existing research progress and the outlook for the future.

4.1. Drugs

Drugs which have been currently approved by the Food and Drug Administration (FDA) in the US include donepezil, rivastigmine, and galantamine are acetylcholinesterase inhibitors. These drugs are molecularly targeted to amyloid and tau proteins and are effective in patients who are in the mild to

moderate stage of AD [71]. Memantine, an NMDA receptor antagonist, can treat moderate to severe AD by diminishing the neuron excitotoxicity due to excessive glutamatergic transmission. In addition, several drugs targeting cholinergic or glutamatergic neurotransmission have been approved, but they can only have a palliative effect [72]. Numerous recently developed drugs are still in clinical trials, but unfortunately, many of them have been declared as failures because they are toxic to other parts of the body, or have no effect on the improvement of cognition or slowing down the development of AD [73].

4.2. Gene Therapy

Research has shown a close correlation between the APOE gene, presenilin 1 & 2, mutations in β amyloid precursor protein and the pathogenesis of AD [74,75]. The result demonstrates that gene mutations contribute to the pathogenesis of AD, reparing the gene defect could potentiall prevent the onset of AD [Khan]. Katsouri, L. et al. successfully injected Peroxisome proliferator-activated receptor gamma coactivator 1-alpha using a viral vector [76]. Rafii et al. demonstrated the feasibility of Adeno-associated viral vector (serotype 2)-nerve growth factor (AAV2-NGF) delivery, but gene targeting is still needed to derive clinical results [77]. More therapeutic options for AD centered on gene editing technology are still under investigation.

5. Conclusion

As a greatly lethal and complex neurodegenerative disease, AD has attracted the attention of scientists around the world. Research on the pathology and pathogenesis of AD is centered on Aβ accumulation, which interacts with other biomarkers to cause oxidative stress, neuroinflammation, mitochondrial dysfunction, synaptic dysfunction and other adverse effects, leading to further deterioration of the disease. PET scanning has shown promise in the clinical confirmation of AD, and tracers adapted to different biomarkers are being updated in research. MRI and fluid testing also play a supporting role. The development and experimentation of targeted drugs have never stopped, and gene therapy has shown unique advantages. Understanding specific pathogenetic mechanisms of AD under all kinds of biomarker, improving the defects of diagnostic tools, and continuing the search and experimention for new therapeutic tools will be the future direction of research.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230837

Trigger of immune deficiency lead to fungal infection in animal

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Abstract. The immune system, as an important protection for animals and humans, ensures that they are not attacked by invading microorganisms and destroying them. Once immune system is damaged or defective, it allows harmful microorganisms to grow and spread within the body, attacking internal organs, leading to infections and diseases. When animals are infected with fungi and cause diseases, they can be treated with antibiotics or by surgery. However, probability of diseases caused by fungal infections in animals has gradually increased. Fungi, as a type of microorganisms, play a dominant role in fungal diseases. Therefore, in the article, it will study how microorganisms operate in animals to cause infection, and lead to fungal infectious diseases through analyzing the mechanism of microbial fungi in animals with immune system defects and their impact on animals. Many studies have shown that the common fungal pathogens of infectious diseases exist in the environment of daily life and inside an animal's body. Poor living conditions or improper feeding are the reasons that trigger the growth of microbial fungi in animals. Through research results, improving animal immunity, creating a good living environment and diet can prevent animals from being infected by fungi and leading to diseases. This series of movements of fungi in animals also reflects importance to both the animal body and the environment. Research on the mechanisms of various microorganisms in infections and diseases can also facilitate development for faster and more effective treatment methods, thereby reducing animal infection caused diseases.

Keywords: fungal infection, immune deficiency, aspergillosis, mucormycosis, candidiasis.

1. Introduction

Not only will humans get infected by fungi and cause diseases, but this can also happen in animals. Microorganisms and fungi that present in the environment do not have an impact on healthy animals most of the time, but they can be harmful in unhealthy animals. Like humans, animals also have an immune system in order to protect their body. Unhealthy animals are often accompanied by immune deficiencies. Not only on poultry, but also pets raised at home may be infected with fungi. Some diseases can even be transmitted between animals and humans. Even more severe cases may lead to their death. Disseminated aspergillosis is one of the common infectious diseases among dogs. When fungi enter a dog's body, they are able to attack organs throughout the body, leading to the disease. This infectious disease is also highly fatal in dogs. In poultry, if pregnant cows are infected with Aspergillus, it will lead to miscarriage or affect the newborn calves with skin infection [1]. Such diseases can usually be treated with antibiotics. Topical treatment is the most common method, with a

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DOI: 10.54254/2753-8818/21/20230837

success rate of 80%. Surgery can also be added to remove the infected part. Although Aspergillosis can be alleviated and treated with antibiotics, the different types of antibiotics will have various effects. The degree of treatment and medical costs also vary. Even so, the 100% successful treatment rate is still not guaranteed [1]. Blastomycosis is also a fungal infection that easily infects cats and dogs. Mainly developing and spreading in the lungs of animals. The most susceptible areas are the lungs, skin, respiratory tract, and eyes, developing nodules. Antibiotics are also preferred method for treating blastomycosis, but they also cannot guarantee a 100% cure rate and there is a possibility of recurrence. Some animals may even be affected by the drug again. At the same time as reducing costs, the treatment cycle will also be extended [2]. Currently, the most common method of treating fungal infections is through use of antibiotics. Although there are treatable methods, they may not be able to clean the pathogen and there is still a possibility of recurrence. The cost and cycle of treatment are also closely related, which has become a major challenge in treatment. Identifying the causes that trigger and give fungi the opportunity to affect animals can prevent them from getting sick, thereby reducing the incidence of fungal infections in animals. Microbial fungi play a main role in diseases caused by fungal infections. Most animals infected with fungi have immune deficiencies that give fungi the chance to cause disease in the body. By analyzing infection cases of different fungi, aim to investigate how fungi can colonize in low immunity animals and spread of fungi within the body and ultimate impact to help prevent infection.

2. Microorganisms in fungal infectious diseases

Microorganisms play an important role in many infectious diseases. Although some infectious diseases caused by fungi are relatively uncommon among animals and humans with healthy physical function and strong immune systems. But in the past few decades, more and more fungal diseases occur in animals, which makes the study of fungal infectious diseases of animals more significant [3]. Some fungal diseases can spread between humans and animals. Animals most susceptible to fungal infections are usually those with weak or defective immune systems. When fungi enter an animal's body, they can spread to various organs. Once they are able to develop and affect organs, it can lead to different types of diseases. Candida albican, the fungi that causes Candidiasis, which can cause diseases in the gastrointestinal tract, mouth, lungs, skin, and heart in birds. In dogs, it can cause inflammation in their skin and organs [3]. The Aspergillus that causes Aspergillosis can also cause inflammation and infections in animal’s on the eyes, bronchi, intestines, mammary glands, and lungs. There is also a type of fungal infection that can infect different parts of an animal's body. Mucorales in mucormycosis enter a dog's body and cause infections, it can affect their gastrointestinal tract, skin, and blood vessels. If not treated promptly, it will lead to dog death. Deficiencies and declines in the immune system give fungi chances to grow and disturb the operation of other functions in the animal's body, which leads to the development of diseases [3].

Aspergillosis is one of the fungal infectious diseases that cannot spread between animals and humans. It’s mainly caused by aspergillus and covers a wide range of types of diseases. Fungi are usually not considered as pathogens because they are ubiquitous in the environment, including Aspergillus. The spreading of Aspergillus is through spores produced by Aspergillus entering the animal's body [4]. Fungi under the form of spores have stronger survival ability, vitality, and higher transmission ability. Animals with healthy physical functions will not be affected although they consume a certain amount of spores [5]. It usually targets animals with weakened immune systems. Phagocytes in the immune system play an important role in protecting the animal body from attacks by harmful cells, microorganisms and other pathogens, and can remove harmful foreign bodies. Among Phagocyte cells, there is a white blood cell called a neutrophil, which has significant effects in the composition of the immune system and is the first “gate” to pass when pathogens enter the body. Since neutrophils belong to Phagocytes, they also have phagocytic capacity. Neutrophils will cooperate with other phagocytes and macrophages to destroy Aspergillus spores at the infection site, preventing further spread and infection of fungi [6]. Therefore, when Aspergillus spores enter an animal body with a strong immune system, they will be destroyed by phagocytes. But if it’s an animal body with

DOI: 10.54254/2753-8818/21/20230837

low immunity, it will be accompanied by a decrease in neutrophils, and Aspergillus fungi can have the opportunity to grow in the body and affect other organs. According to reports, an infection caused by Aspergillus flavus accounts for 10% of Aspergillosis [7]. Aspergillus flavus is a type of corrosive fungi with a complex structure consisting of linear mycelium growth. It has faster growth ability and pathogenicity. Compare to the growth environment requirements of other ordinary fungi, the suitable temperature for Aspergillus flavus to growth is approximately 37 degrees Celsius, while the most suitable grow environment for ordinary fungi is between 25-42 degrees Celsius [8]. This adaptation to environmental conditions creates Aspergillus flavus pathogenicity and explains their ability to grow rapidly and cause damage in the animal body. The largest population is affected by Aspergillosis in birds, which is also one of the fungal infections with a high incidence and mortality rate among birds. In addition, their immune deficiency makes them unable to resist the ingestion of spores and grows in the body, producing toxins that damage organs in various parts, and even causing the growth of nodules. Environmental factors are also an indirect cause of the infection. The hygiene issues might cause Aspergillus in the soil to enter the bird's body, they can also adhere through the feed. If the hygiene of the environment and feed are not done properly, it's likely to further lead to the outbreak of Aspergillus and the infection of diseases [4]. Overall, microbial Aspergillus can have a significant impact in the environment and when entering an animal's body with low immune capacity, leading to the development of diseases.

Mucormycosis is also a type of fungal infection that has no infectious ability between animals and humans. Animals with immune deficiencies also have a higher probability of infection and illness. It's caused by the fungi called mucorales. Mucorales also exist widely in the environment, usually in soil, belonging to environmental organisms. Mucorales are rarely seen as pathogens for healthy animals and generally do not cause diseases due to their low toxicity. The spread and growth of mucorales are similar to Aspergillus, both of which propagate in the form of spores, and it's also a type of corrosive fungi [9,10]. The neutrophils in the immune system also can control Mucorales's function in the animal's body. When Mucorale enters the animal body with a healthy immune system, the neutrophils can damage and engulf the fungal structure of Mucorale, preventing their further infection and growth [11]. So if the immune system function of an animal is weakened and accompanied by the loss of neutrophils, it will cause further reproduction of Mucorales in the body, which cannot be controlled. The suitable environment for the growth of mucorale is around 25 - 37 Celcius, which is a heat-resistant fungus that sustains its ability to grow in the body. The characteristic of the mycelium of Mucorales can rapid growth and have various types of structure. The spore sac growing at the top of the mycelium can release spores for reproduction. Another characteristic of Mucorales is that they could obtain iron from the host they are attached on. Fungal pathogens can get iron from the host's hemoglobin. The most common site of fungal infection is in the blood vessels. They will attack blood vessels, leading to blockages and necrosis within it, as well as the formation of blood clots. The necrosis of cellular tissue and the resulting blockage of blood vessels can affect the normal operation of white blood cells and their inability to resist fungal invasion. This may even lead to the failure of other organs that transport blood to other parts of the body [11]. Mucormycosis also has a significant impact on dogs. When dogs with weakened immune systems are infected by Mucorales, they are able to rapidly and extensively reproduce in their skin, gastrointestinal tract, and other areas. The invasion of blood vessels gradually leads to a decrease in blood flow in the blood vessels, ultimately cutting off all blood vessel pathways, and damaging the body's tissues. If not treated in a timely manner, it will lead to the death of the dog [12]. Although microbial Mucorale exists in the living environment, once animals are affected by it and have the opportunity to grow and infect in the body, it will ultimately pose a threat to animal life.

Candidiasis is one of the most common fungal infections within animals. The cause of the infection is a pathogen called Candida. Candida usually exists by small amounts in the body, gastrointestinal tract, and oral cavity of animals, it's generally not considered a pathogen. But if the immune system or normal digestive microbiota are disrupted, it will increase the probability of contracting Candidiasis [13]. One of the main causes of Candidiasis is Candida albicans. In most cases, the presence of

DOI: 10.54254/2753-8818/21/20230837

Candida albicans in the body does not cause harm to the body or affect animal bodies, leading to disease. But at the same time, they are able to cause varying degrees of impact within the body, and even lead to a life crisis. In healthy animals, the pathogenicity of Candida albicans is not apparent, but in animals with immune system problems, the pathogenicity of Candida albicans is reflected. The range of species infected by Candida albican is very diverse due to their variable attributes, which enable them to quickly adapt and survive under different conditions. When the yeast cells in Candida albican have the opportunity to combine with the cells on the attached host, the fungi will change from the form of yeast to mycelium for growth and invade through phagocytes. Yeast cells can also generate biofilms, covering them below, while mycelium cells grow on top of the biofilm. Biofilm is formed by some of the most common substrates, and the maturity of the biofilm means that Candida albicans have much stronger resistance to attacks and clearance from the host immune system. Such a transformable appearance gives it flexible mobility. Not only does Candida albicans undergo morphological changes when in contact with cells invading their host, but they can also adjust and adapt to changes in environmental factors. When at low pH concentrations (<6), Candida albicans will survive and grow in the form of yeast. When at high concentrations of pH(>7), Candida albicans will grow in the form of hyphae. In addition to its powerful form transformation ability, Candida albicans also have different mechanisms for attacking invading animals. Candida albicans express specialized invasin proteins on the surface of host cells, when combined with host ligands, induce host cells to engulf fungal cells. Although fungal cells are engulfed and appear to be killed, they are actually absorbed. During this process, fungal cells do not need to have excessive activity, but rather passively produce effects. The strong stress response exhibited by Candida albicans in adapting to the environment has contributed to its survival ability and virulence. The constantly changing external conditions and simultaneous invasion of the host are the main reasons why Candida albicans can settle and grow in the host body[14]. Through series of infections of Candida albicans in animals eventually lead to disease, which shows that the microorganism Candida albicans has a great impact on animals in causing fungal infectious diseases.

Microorganisms are inseparable in daily life, not only affecting animal and human health, but also being one of the often overlook causes of diseases. When microorganisms enter an animal's body, they have the opportunity to grow and reproduce, it will affect the animal's body and infect it, ultimately developing into a disease. Some diseases can even threaten the lives of animals. In addition to environmental factors, hygiene and the food they come into contact with can promote the entry of microorganisms into the body, leading to infection. The deficiency of immune function is also one of the important reasons that fungi have the opportunity to spread and continue to grow in the body. A healthy immune system can destroy and engulf harmful microorganisms, viruses, and cells that enter the body. Being able to further prevent their continued diffusion within the body. When there is a problem with the immune system, invading microorganisms cannot be cleaned up or distinguished from normal cells, which can cause them to continue to spread and ultimately lead to diseases. The best way to prevent the impact of diseases is to increase and protect the immune system of animals, enabling them to have the most basic self-protection ability. The study on antibiotics can also be on the mechanism of microorganisms in animals. Clearing the source of infection before further severe infection can reduce the pain and mortality suffered by animals.

3. Conclusion

According to the analyzed cases, immune deficiency is the main reason for fungal infections in animals. Animals with immune deficiency cannot resist and eliminate fungi that enter the body properly. Fungi that enter the body of animals can spread through their movement mechanisms, and some fungi can enter and spread to any part in the body. If detected early, it can be treated by using antibiotics. However, the efficacy of different antibiotics varies, the probability of successful treatment cannot be guaranteed also. The cost of treatment also depends on which type of antibiotic is used. If not treated in a timely manner, it can lead to the spread of fungi and have a greater impact, which can lead to animal death. By analyzing the mechanism of action of microorganisms and fungi in animals,

the importance of microorganisms in disease research and treatment cannot be underestimated. Microorganisms are able to spread in the body due to the inability of the immune system to function properly and warn that a healthy immune system is the most fundamental protection against any infection. Although there is currently no guaranteed treatment of fungal infection that can fully cure animals. Further research on microorganisms can help in finding more effective treatments and drugs. The importance of animal protection and health can help animals stay away from the troubles of diseases.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230839

Revolutionizing vaccinology: The rise of mRNA vaccine

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Abstract. The public health sector has been greatly affected by the COVID-19 pandemic, and vaccines have become a crucial means of managing the virus. mRNA vaccines have gained prominence among the available vaccines due to their effectiveness and safety, marking a significant advancement in the field of biotechnology. Traditional vaccines have often resulted in severe symptoms and partial protection, while newer recombinant vaccines have significant drawbacks. In contrast, mRNA vaccines offer unprecedented cost-effectiveness, safety, and high efficacy per dose. The mRNA vaccines transport bioengineered mRNA to the human body, which translates into proteins that stimulate the immune response. However, there are some challenges associated with the development and production of mRNA vaccines, such as efficient delivery and maintaining the integrity of the mRNA molecule during storage and delivery. Despite these challenges, mRNA vaccines represent the future of vaccinology and the first line of defense against major infectious diseases. This paper explores the structure, mechanisms, immunology, and related areas of study concerning mRNA vaccines. The paper emphasizes the challenges in developing and producing mRNA vaccines and potential solutions to overcome these challenges.

Keywords: mRNA, vaccines, COVID-19 pandemic.

1. Introduction

As the COVID-19 pandemic swept across the globe, healthcare professionals and medical experts increasingly relied on vaccines to contain the spread of the virus. Notably, the pandemic witnessed the development and licensure of the first mRNA vaccines, heralding a new era in immunology. It is worth noting that the first conventional vaccine was developed by Dr. Edward Jenner in 1796. Dr. Jenner discovered that individuals with cowpox did not contract smallpox and subsequently inoculated an eight-year-old boy named James Phipps with a crude vaccine. This event marked the first successful human vaccination, resulting in Phipps' immunity to smallpox [1]. Since the development of the first vaccine, vaccination technology has evolved considerably, with enhanced efficacy and fewer side effects. Conventional vaccines, including live, live-attenuated, or killed vaccines, have often resulted in severe symptoms while only providing partial protection. In contrast, newer recombinant vaccines, such as DNA, subunit, and virus-like particle vaccines, have demonstrated improved efficacy but have significant drawbacks, such as the need for complementary adjuvants to maintain efficacy and the potential for adverse effects. During the COVID-19 pandemic, mRNA vaccines demonstrated their revolutionary potential in biotechnology, offering unprecedented cost-effectiveness and safety. Among the key benefits of mRNA vaccines are their excellent safety profile, ease of production, high efficacy

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DOI: 10.54254/2753-8818/21/20230839

per dose, flexibility in adjuvant usage, and rapid response time to emerging variants. mRNA vaccines transport bioengineered mRNA to the human body via various transportation vessels or as a naked vaccine. Once inside the body's cells, the mRNA translates into proteins, which express themselves on extracellular major histocompatibility complex (MHC) complexes and stimulate the human immune response [2]. However, there are some challenges associated with the development and production of mRNA vaccines. One of the main challenges is the requirement for an efficient delivery system, which can be achieved by using lipid nanoparticles. Besides, the lipid nanoparticles used for mRNA vaccine delivery may result in potential side effects. Another challenge is the need to maintain the integrity of the mRNA molecule during production, storage, and delivery, which can be influenced by factors such as temperature, pH, and enzymatic degradation. Despite these challenges, mRNA vaccines represent a major breakthrough in the field of vaccinology. Overall, mRNA vaccines represent the future of vaccinology and the first line of defense against major infectious diseases. This paper will explore the structure, mechanisms, immunology, and related areas of study concerning mRNA.

2. Different types of mRNA vaccines

mRNA vaccines are a new type of vaccine technology that has gained significant attention during the COVID-19 pandemic. They have demonstrated high efficacy and safety profiles and have quickly become the cornerstone of vaccination efforts worldwide. There are three main types of mRNA vaccines: non-replicating mRNA, self-amplifying mRNA (saRNA), and circular RNA (circRNA). (Figure 1)

Non-replicating mRNA is the most widespread and maturely developed type of mRNA present in mRNA vaccines. Similar to natural mRNA, non-replicating mRNA encodes the target antigen and has 5 prime and 3 prime untranslated regions (5’ and 3’ UTRs). These regions help in the stability and translation of the mRNA molecule. Non-replicating mRNA is translated into the target protein antigen by host cell ribosomes and is then presented on the extracellular membrane via MHC I and II complexes, leading to an immune response [3-4].

saRNA is an alternative to non-replicating RNA that contains not only the genetic information for the virus’ antigen but also the information for replication. This genetic code allows saRNA to replicate within the cytoplasm of the host cell. Intracellular replication comes the benefit of increased mRNA translation efficiency and extensive protein expression, resulting in higher efficacy compared to non-replicating RNA. Compared to traditional non-replicating RNA vaccines, the ability of saRNA to self-amplify gives it the advantage of producing higher levels of viral antigen of vaccine origin, allowing for a lower dose, and looser requirements for delivery system efficiency [5].

In terms of safety, both saRNA and non-replicating RNA vaccines have risks for excessive inflammation, but of different causes. Lasting saRNA and continued production of antigen could imbalance the normal function of host cells. Comparatively, unmodified non-replicating mRNA may elicit a type-I interferon response, resulting in critical inflammation. Due to the requirement for an efficient delivery system, components built by lipids and nucleosides of foreign origin may result in potential symptoms [5].

Circular RNA (circRNA) is a newly discovered type of mRNA that requires additional study and refinement. This form of non-coding RNA has a unique looped structure that provides increased structural durability and greater resilience against degradation when compared to other types of mRNA. The intricate secondary structure of circRNA may impact the efficiency of the vaccine. Advantages of circRNA over any linear RNA vaccine include a more relaxed requirement on transportation, storage, and production environments due to the closed-loop structure of circRNA. This allows the vaccine to be transported and stored in a wider variety of locations, potentially lowering the cost of production. SARS-Cov-2-targeting circRNA vaccines have demonstrated the capability to produce potent and functional viral antigens, making it a promising vaccine technology against infectious diseases [6-7].

mRNA vaccines have revolutionized the field of vaccinology and have significant advantages over traditional vaccines. They provide high efficacy per dose, a desirable safety profile, ease of production,

DOI: 10.54254/2753-8818/21/20230839

and high flexibility with regard to adjuvant usage. mRNA vaccines are the future of vaccinology and will serve as the first layer of armor in the defense of major infectious diseases.

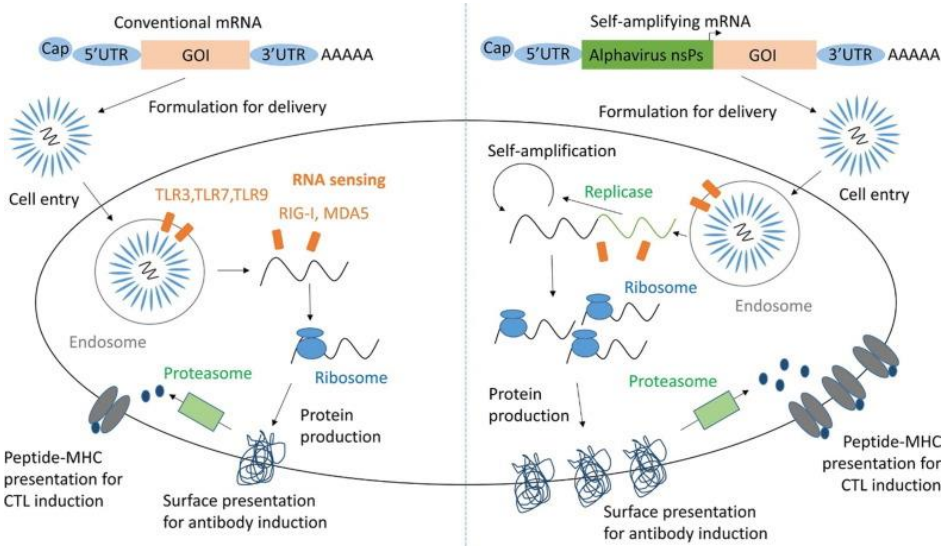

Figure 1. Structure of different types of mRNA [4].

3. mRNA vaccines macunfacturing

mRNA vaccines have become a critical tool in the fight against infectious diseases, and their production process has been the subject of intense research and optimization. The mRNA manufacturing process involves two main steps: upstream processing and downstream processing, followed by a final packaging process. The upstream processing step involves the production of mRNA utilizing RNA polymerase and subsequent enzymes, while the downstream processing step involves the purification and quality control of the produced mRNA. A final packaging process follows, where mRNA is packaged into lipid nanoparticles or alternative delivery vessels.

The manufacturing process plays into the superior safety profile of mRNA vaccines. As the production process excludes cell cultures and any unprocessed materials of animal origin, mRNA vaccines do not include lipopolysaccharides, cellular or foreign impurities, making it safer. Compared to other complex biologicals, the short manufacturing period further lowers risks of contamination.

Upstream processing constructs the mRNA of interest with in vitro transcription (IVT). IVT depends on a series of reactions between RNA polymerases, a linear DNA template, and nucleotide triphosphate substrates. T3, T7, or SP6 RNA polymerases are utilized to catalyze the manufacturing of intended mRNA strands from the previously produced complementary linear-DNA template in IVT.

There are two distinct ways of approaching mRNA capping: The first capping process is executed at the time of IVT, replacing a fraction of the guanosine triphosphate with a cap analog. An alternative capping process involves establishing an independent enzymatic reaction, employing the vaccinia capping enzyme (VCC) and a methyl-donor substrate to cap the mRNA. This alternative capping method guarantees a successful cap with higher efficiency.

The purification process is vital for the mRNA vaccine to meet clinical-grade purity standards. Enzymatic compounds, nucleotides, nucleosides, and DNA templates must be excluded from the final product to avoid triggering acute host immune responses. The purification process has a significant impact on the economy of the entire production process, as the downstream process is a delicate balance between mRNA quality and cost.

The cost of manufacturing mRNA vaccines is largely dependent on the production strategies. Further research and optimization are needed for a more cost-efficient production system. The costs of five prime cap analogs and modified nucleotides also play a role. Essentially, expenditure concerning

DOI: 10.54254/2753-8818/21/20230839

mRNA production has significant correlations with the quantity of RNA per dose of vaccine, production scale, and production titers.

In conclusion, the optimized manufacturing process has played a significant role in the success of mRNA vaccines, with the process being safe and highly specific. Further research and optimization of the production process will likely lead to more cost-effective and widely accessible vaccines [8].

4. Mechanism of mRNA vaccines

The mRNA vaccine has indeed shown remarkable efficacy in combating the COVID-19 pandemic. However, as the virus continues to mutate, it is important to understand the underlying mechanisms of the mRNA vaccine in order to further develop and modify it.

The mRNA component of the vaccine is crucial in inducing an immune response, and selecting the appropriate antigen is key to producing effective antibodies against the targeted virus. In the case of the SARS-CoV-2 vaccine, the mRNA contains the full sequence of the spike (S) protein, which is found protruding from the surface of the virus and is responsible for recognizing and fusing with host cells. The genetic sequence of the antigen is modified with prolines to increase its stability [8].

Once the mRNA vaccine enters the host cell, it is released from the lipid nanoparticles and is translated by ribosomes to form proteins. As showing in Figure 2, these proteins can take two different paths: they can either be fragmented by proteasomes and expressed as the antigens that delivery to the rough endoplasmic reticulum and are captured by MHC class I peptide, presenting to $CD8^+$ T lymphocytes; or they can be expressed as exogenous proteins which will be carried by maturing antigen-presenting dendritic cells and travel to lymph nodes.

B cells will be further stimulated to produce antibodies that target the antigen encoded in the mRNA strand. These free-moving antibodies are the key to achieving immunity against antigenic infections. The $CD8^+$ T cell immune response is instantaneous, while the B cell response takes longer to develop and provides long-term protection [9-10].

Understanding the underlying mechanisms of the mRNA vaccine is crucial for further development and modification to combat evolving viruses. The selection of antigens, optimization of the manufacturing process, and exploration of new delivery systems are all areas of ongoing research in the development of mRNA vaccines.

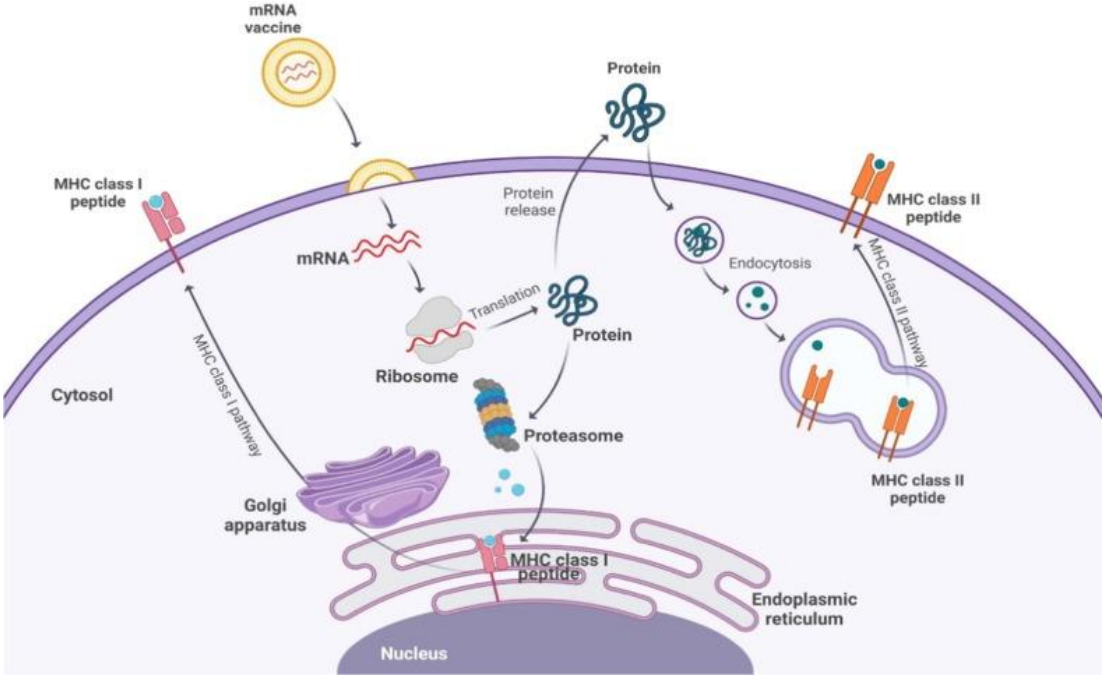

Figure 2. Schematics of mRNA vaccines stimulating immune response path [2].

DOI: 10.54254/2753-8818/21/20230839

5. Conclusion

mRNA vaccines have proven to be highly effective in combating the COVID-19 pandemic. However, there are several limitations that are hindering their widespread use. One major setback is the instability of the mRNA molecule, which is highly fragile and prone to rapid degradation if exposed to temperatures outside of its preferred storage conditions. This limits the real-world applications of mRNA vaccines, as maintaining the necessary storage conditions is economically unachievable in many regions around the globe.

Another critical weakness of mRNA vaccines is the relatively high dosage volume and the need for several doses to achieve bodily immunity. This places a high demand for patient compliance, which can be challenging to achieve in various political areas globally. Patients often have to pay out-of-pocket for vaccines, and market pricing plays a significant role in limiting accessibility. The cost of production, facility, research and development funding, transportation, storage, commercial, marketing, and profit margins all contribute to the final price. Estimates put the cost of the Pfizer vaccine at $38 per patient, while the Moderna vaccine ranges from $30 to $50. These costs can be prohibitive for many individuals, particularly in regions where public sector assistance is limited.

Furthermore, compared to other vaccines, the out-of-pocket cost for patients is still somewhat higher, limiting accessibility to the vaccine. As these vaccines travel further from their manufacturing plants, their prices only increase. This places additional burdens on individuals who are left out of insurance or government care, making it more difficult for them to attain doses of the vaccine.

The limitations placed on mRNA vaccines are preventing them from becoming an accessible pharmaceutical that is highly capable of immunizing patients. Research on stabilizing mRNA structures and superstructures that can help mRNA molecules obtain higher structural integrity can solve many of the issues listed above. Maturing the production pipeline can also help reduce the manufacturing costs of mRNA vaccines, making them more accessible to a wider population.

Overall, mRNA vaccines represent a revolutionary idea of what a vaccine can achieve. They bring innovation to biotechnology, and there are high hopes for their potential in treating and preventing other diseases. However, until the limitations discussed above are addressed, their widespread use will remain limited. It is crucial to focus on finding solutions to these limitations to ensure that mRNA vaccines can reach a broader population and have a significant impact on global public health.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230842

The pathogen, clinical overview and influence of depression

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Abstract. Depression is a mental health disorder that affects a significant portion of the global population. At the same time, the influence of depression on a person's physical and mental health is well established and studying the impact of depression on the outcome of a pathogen can lead to improved healthcare outcomes for individuals suffering from both. So, this article focuses on the pathogen, clinical overview and influence which mainly gathers information from different aspects to give a summary of areas of depression based on the data and results from recent studies. The research results indicate that depression is a complex disease that encompasses different causes, including genetic and biological factors. The recurrence of depression is a common phenomenon, and people with a history of depression should be more cautious, although drugs targeting recurrence have emerged. When early warning signals are detected, patients need to take action to prevent them.

Keywords: depression, situation, relapse, medical treatment, types.

1. Introduction

The characteristic of depression is persistent feelings of sadness, hopelessness, and a lack of interest in previously enjoyed activities. It is estimated that the number of individuals experiencing depression has increased globally, highlighting the need for continued efforts to address this pressing issue. Electroconvulsive therapy (ECT) and mixed therapy are coming up since there has been a growing recognition of the importance of mental health and the impact of depression. Also, there has been a growing emphasis on preventative strategies and early intervention, with a focus on improving access to care associated with mental health issues. Based on the development of technology in mental health aspect, researchers have found new and more effective ways of treatment and giving out more problems in different areas of psychology. This article gives an overview of depression, including new findings and definitions related to it. The article classifies and organizes the information from journals and gathers it together. So, this article is mainly focused on giving some basic information about depression. The research results of this article are beneficial for those who do not have much knowledge of depression to understand more about the current situation of depression and related treatment and recurrence, so as to better pay attention to their own health.

2. Causes

Depression could be caused by several factors. The most common reasons are social failure, family effects, pregnancy, and so on.

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DOI: 10.54254/2753-8818/21/20230842

Everyone in daily life needs to face different people and make different social contacts. Some people may feel failed during social contact. In the report of Liu et al., the main symptom of depression is losing happiness and pleasure in daily life. Figure 1A shows the social defeat group (n = 9) and the control group (n = 8) had distinct variations in the main components analysis score plot produced from GC-MS spectra, it clearly shows the differences between the social defeat group and control groups (R2X=0.409, Q2= 0.066). The OPLS-DA model, shown in Figure 1B, demonstrates good prediction between the social defeat group and the control group. 25 metabolites were significantly differentiable between the two groups based on the OPLS-DA (R2X = 0.370, R2Y = 0.793, Q2 = 0.151). Figure 1C reveals the permutation plot demonstrated the validity of the first OPLS-DA model. Based on the outcome of the experiment (as shown in Figure 1), when male Sprague Dawley (SD) faces social failure, the molecular differences in samples from the two groups. SD's brain changed, and 25 different metabolites were significantly less than in the control group [1]. Socially defeated rats have lower scores on the sucrose preference test, which means they have more reactions and are more likely to have depression. Mainly, the disruption of lipid metabolism, amino acid metabolism, and energy metabolism in the prefrontal cortex of socially defeated rats causes depression-like behaviour.

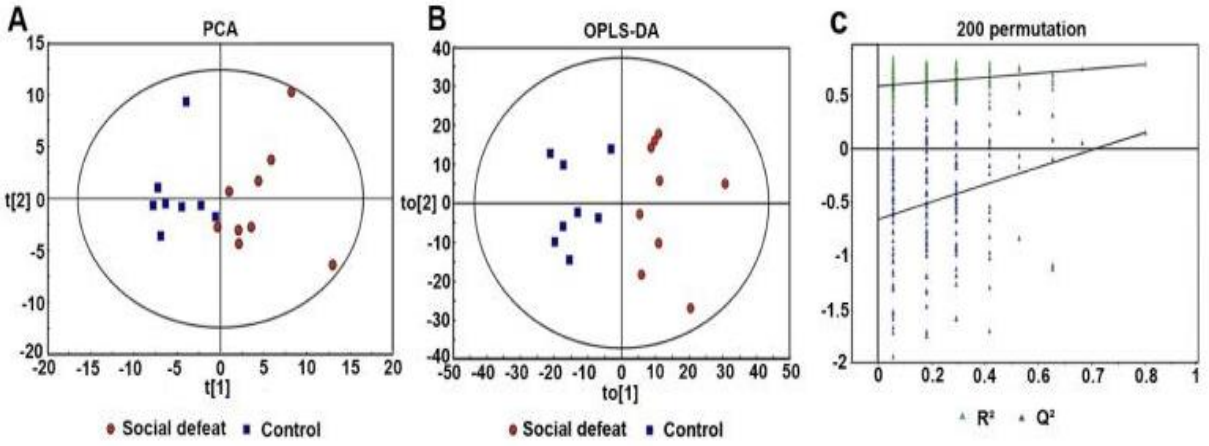

Figure 1. Metabolomics analysis of the prefrontal cortex [1].

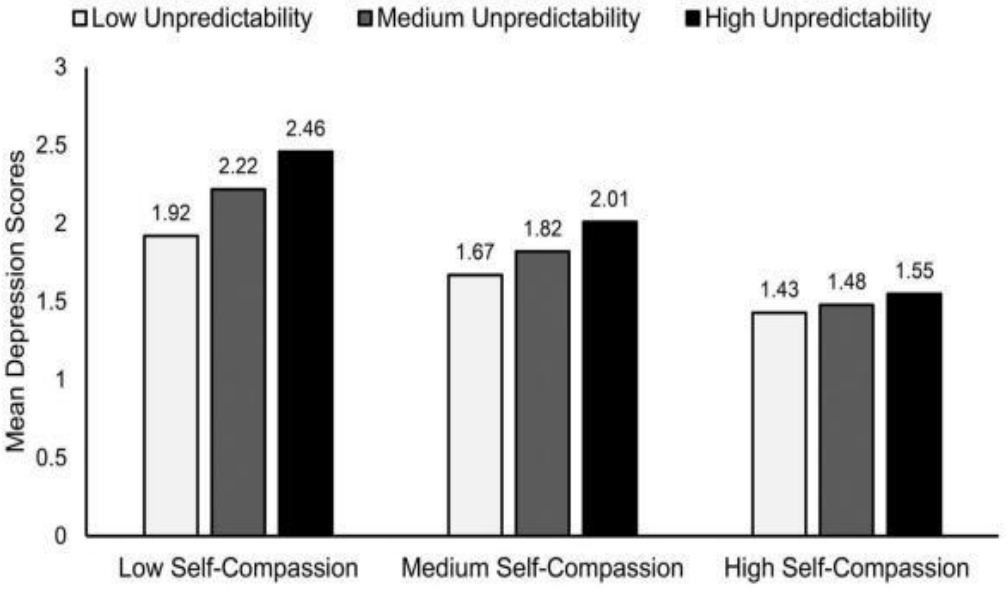

Figure 2. Degree of self-compassion between family unpredictability and recent depression [2].

DOI: 10.54254/2753-8818/21/20230842

During adolescence, teenagers need to face strange world environments and explore knowledge, the family environment becomes much more critical. According to Hood, Thomson Ross, & Wills, family unpredictability includes unsteady meals, money or income, parental control and parental nurturance, and all of them are related to depressive and anxiety symptoms in kids [2]. Another factor is self-compassion. Between self-pity, depression, and family unpredictability, high unpredictability is associated with high depression scores. High self-compassion is associated with low depression scores. This means that when faced with varying degrees of family unpredictability, self-compassion can also affect a person's depression score (as shown in Figure 2). So, less support from family leads to more unpredictable and frequent depression symptoms. On the other hand, training self-compassion among college or university students can decrease the probability of depression.

3. Types

In daily life, the most common form of depression is caused by failure. But depression is not a word with a specific disease; there are also a large number of depression types. Except for the depression caused by daily failure, there are two other types of depression that are also very common: depression in pregnancy and Seasonal Affective Disorder (SAD).

Depression in pregnancy has two types, antenatal depression and postnatal depression. In a study in Turkey, 71 (27.5%) of 258 people had prenatal depression [3]. The experiment used the Multidimensional Scale of Perceived Social Support (MSPSS) to evaluate the social support of pregnant women; The Edinburgh Postpartum Depression Scale (EPDS) is used to measure the collected data. According to EPDS, the score is irrelevant with gestational age. It shows a positive correlation with age and is related to the rate of pregnancy and the rate of living children.

Another type of depression that is not well known is called Seasonal Affective Disorder (SAD). This is usually seen in winter, and remission occurs in spring or summer. The common symptom of SAD is the opposite of classical depression. Classical depression shows loss of weight, sleep, and appetite, but SAD is shown to increase appetite, weight gain, and sleep. Most SAD patients crave carbohydrates, and it is mostly shown in the afternoon and evening. Most studies have found SAD to be prevalent in women and children, but it is less common in adults. One reason for SAD in winter is the lack of light. SAD is popular in higher northern latitudes [4].

4. Therapeutic methods

There are different types of medicine to treat depression. The most popular type is selective serotonin reuptake inhibitors (SSRIs). Some popular medicines such as Prozac and Zoloft, are both SSRIs. SSRIs are helping to increase the level of 5-HT in the brain, and they have fewer side effects, but those side effects will improve over time, so it usually considers to be safer medicine. Tricyclic antidepressants are used to cure patients with Moderate to severe depression, they raise the level of 5-HT and norepinephrine in patients' brains, and it also consider to be a safe medicine and the side effects always improve after 10 days (about 1 and a half weeks), but the side effects are serious than SSRIs. Vortioxetine is used to cure adult patients with recurrent major depressive episodes, but it was only given when patients give no response to two types of medicine before and it has more side effects than the other two such as naupathia, emesis and abnormal dreams [5]. SNRIs (Selective Norepinephrine Reuptake Inhibitors) treat depression by stopping 5-HT and norepinephrine from back to the cell and releasing it to make sure the level of norepinephrine and 5-HT is at a normal level. There are still some slight side effects, but some medicine of SNRIs such as Fetzima and Savella [6]. Some medicine that is not really recommended for treating depression is Monoamine Oxidase Inhibitors (MAOIs), which stop the resolution of 5-HT, norepinephrine, and dopamine to ensure that the level of these chemicals is balanced, but it will lead to blood pressure problems if eaten with other medicine or some typical food, and there are still many side effects for it such as muscle cramps, headaches, weight gain, and so on [7].

CBT (Cognitive Behaviour Therapy), also called talking therapy, is also known for curing depression. It is a kind of therapy that helps deal with the thoughts and behaviour of people. But the way of taking CBT and its frequency will also affect efficiency. CBT-based therapies have better results than other

DOI: 10.54254/2753-8818/21/20230842

psychological and pharmacological therapies, but joint therapies have shown better effects than single therapies. For psychotherapies, the main difference is how the changes achieve instead of the extent of the changes [8]. In metaregression analysis by Cuijpers Huibers, Ebert, Koole, and Andersson show that over ten sessions of CBT, the effect size increased by 0.1, but the effect size and the number of treatments per week had a strong relationship: two instead of one session per week would increase with 0.45 of effect size [9]. The reason is that relationships between patients and counsellors may develop more rapidly when they have more contact and the continued survival of neurons 'born' within the last five days is important for learning to occur. From a clinical point of view, in common, the early stages of therapy should have more intense therapy, especially for patients with severe depression and suicide. In additional metaregression analyses, the total contact time was not found to a high extent related to effect size.

Electroconvulsive therapy (ECT) is a way to cure serious psychiatry including depression, bipolar disorder, mania and so on. ECT has been used for 75 years, but it remains argued out of the psychiatry area due to a misunderstanding of the usage and informed consent process. ECT is considered a secondary treatment when medical treatment fails, or patients show highly suicidal or other clinical symptoms highly suicidal or other clinical symptoms. ECT works by increasing dopamine, serotonergic, and adrenergic neurotransmission. Before doing ECT, the doctor will mention the precautions and get a complete general medical history during the consultation: the illnesses, especially cardiovascular, pulmonary, and central nervous systems must. During the ECT, doctors will check the heart rate, blood pressure and oxygen saturation to ensure everything is going correctly [10]. Comparing ECT with different medical treatments such as MAOI (Monoamine Oxidase Inhibitors) and antidepressants, ECT shows a significant advantage over medical treatment. Even with less relevancy, ECT shows a greater chance of treatment response and less extent than medical treatment [11]. Those demonstrate that ECT is an effective way to cure depression, but it is not clear if it is the primary treatment choice.

Overall, medical treatment is the most common treatment. It has different types of medicine for different degrees of depression. If the single medical treatment did not show very well on the patient, then combine treatment, medical and CBT combine together to expand the effect size to maximize the effectiveness of both two treatments. ECT will only be used when medical treatment and CBT both have less performance, ECT is only used for severe depression.

5. Discussion

Depression causes lots of social influences including social defeat and thwarted belonging. A defeated sense of belonging and burdensome are causes and results of depression, and social anxiety also leads to thwarted belonging. A low level of income is also associated with a prominent level of burdensome, and a defeated sense of belonging and thwarted belonging and burdensome leads to suicide to a large tend [12]. So, depression affects suicidal ideas and is directly associated with suicidal idea. If patients excessively need reassurance, it will cause some meaningful people to keep their distance from patients and let them feel a more defeated sense of belonging. Depressed patients, on the other hand, may feel more burdened by achievement-oriented challenges brought on by exhaustion, experiential avoidance, trouble concentrating, and strained relationships with others.

Some people also believe that in addition to daily behavior and actions, some experiments and environmental changes can affect the impact of unpleasant experiences on brain activity, which may affect the susceptibility to depression. This makes people feel more sensitive to things that happen in daily life, including interpersonal ones. Some neuroscience disorders may decrease activity or levels, and stressful events may affect susceptibility to affective diseases that exist both within and between different neurochemical systems [13]. The research, also clarifies that those changes should not be causal, but secondary than initial daily stressors.

Relapse problem is one of the focus points after cure depression. Evidence suggests that as the illness becomes more frequently recurrent, the risk of depressive recurrence, treatment resistance, and chronic rise. Every year, 5% to 10% of patients who are still taking antidepressants relapse, which has resulted in some surmising tachyphylaxis.

DOI: 10.54254/2753-8818/21/20230842

In one study, which gave patients medicine therapy, cognitive therapy and combination therapy, the data showed that the percentage of relapse in patients who accept cognitive therapy or combined with medical therapy is half less than in patients who continue on medicine [14]. Also, providing cognitive therapy during acute treatment can help prevent depression relapse.

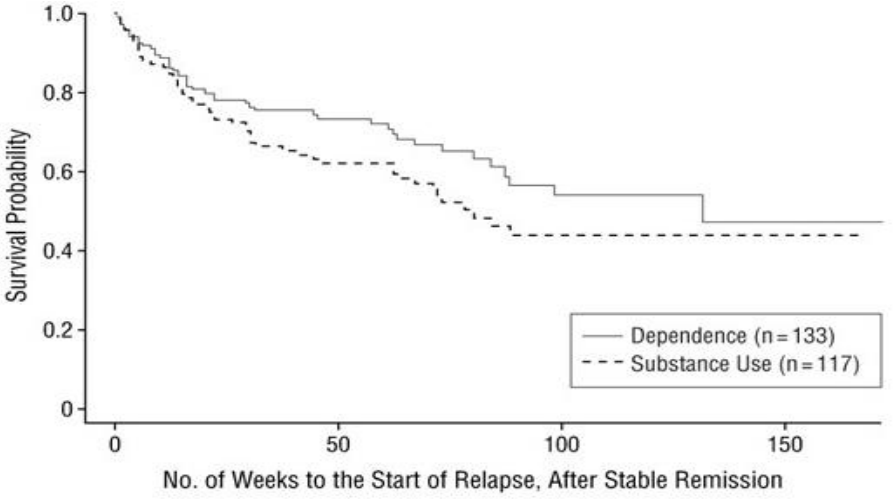

Figure 3. Kaplan-Meier estimates of cumulative probabilities [15].

One of the experiences shows that in 12 months relapse risk periods, 37.1% of patients report relapsing depression symptoms [16]. There are two risk factors for it. One is subthreshold depressive symptoms continuing 7 months after starting antidepressant treatment, and another one is that they used to have more than 2 times major depressive disorder seizure history or persistent mood issues for two years. Another study shows that relapse of depression is also associated with using alcohol, cocaine, and heroin (Figure 3) [15]. For the relapse part, the current study gives out the data and the rate of relapse for different types of treatment and how some external reasons such as alcohol, cocaine and heroin affect relapse of depression and MDD abstinence. For relapse, most of the research did not mention the current relapse rate for different degrees of depression.

6. Conclusion

Depression is a complex disease that has different causes, including genetics and biological factors. Medications for relapse have also been coming up. Relapse of depression is a common occurrence, and individuals with a history of depression should be more careful about it. When identifying early warning signs, patients need to take action to prevent them. The article did not fully address the impact of social and cultural factors on depression. Also, the medical capacity of each community and country does not count in the article. To improve it in the future, the depression area can focus more on the different types of things to measure, and different ways and effectiveness of these methods also need to be considered.

Acknowledgement

I would like to express my thanks for my time and effort. Also, I would like to say thanks to my family for their encouragement and inspiration. It helps me when I feel upset and discouraged, and it played a critical role in my whole article. Finally, I would like to express my gratitude to the University of Southampton, through my study career at the University of Southampton. Throughout my career at the University of Southampton, I learned a lot about psychology and depression to write this article.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230843

From RNA world to RNA-peptide world: A review

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Abstract. How life starts from small molecules to RNAs and further to modern life is an unanswered question. Cyanosulfitic chemistry established the synthesis of building blocks, including 12 proteinogenic amino acids, 4 ribo- and deoxyribo-nucleosides, and phospholipids, from hydrogen cyanide and hydrogen sulfite under prebiotically plausible conditions. Later on, the non-enzymatic monomer extension of nucleotides provided a plausible pathway from mononucleotides to RNAs giving rise to the RNA world. RNA is one of the key components for the origin of life, firstly, the sequence information can be heritage by template copying reaction. Secondly, RNA is able to fold into a secondary structure which has the capability to catalyze chemical reactions. The RNA world scenario has perfectly overcome the chicken-egg problem, but it still cannot explain why peptides are involved in modern life. Most recently, with the establishment of the reaction between RNA and peptides, the trajectory to the RNA-peptide world theory has opened up a new era of the origin of life research. Here I will discuss the current results relevant to the RNA world to RNA-peptide world theory.

Keywords: RNA world, RNA-peptide world, the non-enzymatic replication of nucleotides.

1. Introduction

The source of life on early Earth is still an uncertain question, with a growing body of literature exploring prebiotic building block synthesis like nucleotides and amino acids. For instance, atmospheric hydrogen cyanide can react with surface water and ferrous ions to form ferrocyanide [1], which can then undergo redox reactions to produce precursors for bio-related molecules. This allows for the simultaneous production of all cellular subsystems through a single type of chemistry, with Cu(I)-Cu (II) photoredox cycling and hydrogen sulfide acting as the reducing reagent to accelerate the process [2]. A concentrated cyanide solution could be created by a stream entering the area or a small amount of rainfall. Calcium cyanamide and magnesium nitride can be produced through thermal transformation of calcium and magnesium cyanoferrates (II). These salts can then generate cyanamide and ammonia upon hydration, which are essential for ribonucleotide and purine synthesis, respectively. Common cyanosulfidic chemistry from hydrogen cyanide can synthesize building blocks for RNA, peptides, and lipids [3]. Molecular cell biology has traditionally been dominated by a protein-centric perspective. However, the discovery of tiny non-coding RNAs suggests that this view may be incomplete. Despite their abundance, these RNAs were initially overlooked but have since been found to have an integral roles in controlling expression of gene at numerous points. As a result, they have become recognized as important contributors to the genetics and evolution of organisms [4].

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DOI: 10.54254/2753-8818/21/20230843

2. Noncanonical RNA/DNA copying

The observation of nonenzymatic copying of a number of prebiotically plausible nucleotides has led to the theory that it could have contributed to the formation of RNA as a chemical selection mechanism. If present in the template, nucleotides with changed sugar chemistry, such as arabino-, threo-, and 3'-amino-2'3'-dideoxyribo-nucleotides, can be replicated to generate a canonical RNA strand, although they are less effective than ribonucleotides in nonenzymatic copying. The amino group at the 3'- or 2'-position of the 3'-terminal residue is the primer that is frequently employed for an extension that yields more because of its nucleophilicity, which facilitates the synthesis of phosphoramidate [5]. Primer extension that is Nonenzymatic with triggered arabinonucleotides is significantly less successful than the one with ribonucleotides that are activated. This is according to studies using arabinonucleotides and 2'-deoxyribonucleotides in template-directed primer extension processes. Furthermore, primer extension is strongly hindered by the inclusion of an arabinonucleotide, and primer extension products that have been arabino-terminated cannot be extensively prolonged [6]. These findings support the hypothesis that different copying chemistry's cycles would result in the creation of oligonucleotides consisting numerous ribonucleotides. Further research is needed on the properties and provenance of a potentially primordial form of RNA that contains inosine and 2-thio-pyrimidines [7].

3. Canonical RNA/DNA copying

Although much research has focused on investigating a pre-RNA world, it is important to also consider the possibility of RNA-based RNA replication as a potential hypothesis for the RNA world. The first instances of templated RNA copying were studied by Orgel and his colleagues, who investigated template-directed synthesis on templates with one or more A or T residues within a run of C residues using 32P-labeled hairpin oligonucleotides with oligodeoxynucleotide sequences [8]. More recently, Sosson et al. calculated the rate coefficients for the chemical process of primer extension that involves methylimidazolides or oxyazabenzotriazolides of deoxynucleotides or ribonucleotides. They measured the tie affinity of 15 diverse triggered deoxynucleotides to DNA or RNA templates and provided an explanation for why some primer extensions that are enzyme-free can be copied effectively while others cannot. Effective enzyme-free copying is induced by a grouping of tight binding, quick extension, and slow hydrolysis. They also disclosed the dissociation coefficients for triggered nucleotides binding to their corresponding templates. The rate constants for hydrolysis and the primer extension's chemical process allow for a noble quantifiable alignment between experimentation and theory. This demonstrates that a set of factors, such as the binding coefficients for triggered and inactive nucleotides and the rate coefficients for hydrolysis and the covalent phase of primer extension, is adequate to characterize the reaction of enzyme-free primer extension. To facilitate a more systematic search for RNA primer extensions that provide greater yields, these requirements are best addressed when HOAt esters react with amino-terminal primers [9]. Any of the four ribonucleotides can be incorporated almost quantitatively when an activating reagent (e.g., carbodiimide) and an organocatalyst are combined. In addition to pre-activation chemistry, adenosine 5'-monophosphate was shown to form oligomers in an aqueous solution by in situ activation [10]. Furthermore, the same group evaluated the in-situ activation of all 16 canonical ribonucleotide dimers and two trimers. They discovered that short ribonucleotide oligomers are effective reactants in enzyme-free copying, and that trimers and dimers could be converted to primers on RNA templates using an organocatalyst and optimal condensing agent combination under slightly acidic conditions [11].

Non-enzymatically directed RNA polymerization using activated monomers has been explored for its thermodynamic characteristics since the 1960s. The suggested procedures all start with the template attaching to the activated monomer in a noncovalent reversible fashion. Base stacking that arises from downstream or upstream adjacent mono- or oligonucleotides can facilitate these interactions. The chemical attraction of guanosine 5′-monophosphate (GMP) to the primer-template complex was studied by Tam et al. using isothermal titration calorimetry (ITC) and nuclear magnetic resonance spectroscopy (NMR). Although GMP binding affinity increased by about two orders of magnitude

DOI: 10.54254/2753-8818/21/20230843

when the helper oligonucleotide stabilized the downstream side of the binding monomer [12], the likelihood that GMP binding to the primer-template complex could not pass through the downstream monomer was noticeably increased. In a separate investigation, they showed that using activated helper oligomers did increase rates of nonenzymatic template-directed synthesis. They demonstrate that primer extension can be sped up by employing short 5′-activated oligonucleotides and provide an example of how mixed-sequence RNA templates may be duplicated in a single container. Activated oligomers catalyze the serial addition of activated monomers, producing primer extension products with sufficient fidelity to preserve a genome long enough to form functional ribozymes. Finally, by immobilizing the primer and template on a bead and adding individual monomers in sequence, they successfully produced a large percentage of an active hammerhead ribozyme, creating a bridge between nonenzymatic polymerization and the RNA world.

Using 2-methylimidazole-activated monomers as substrates, Walton et al. explored the impact of varied lengths of downstream auxiliaries. It was shown that downstream oligonucleotides enhance primer extension by creating a high-affinity nucleotide binding site in the 'helper' oligonucleotide's pocket [14]. Using nuclear magnetic resonance (NMR), a unique symmetrical dinucleotide product was identified, which was made possible by a set of activated trimeric oligonucleotides that sped up the successive addition of monomers to primers. Synthetic methods were employed to confirm the presence and catalytic activity of the 2-methylimidazolium-bridged dinucleotide. Non-enzymatic template-directed primer extension with nucleoside 5'-monophosphate imidazolides entails two steps: the formation of the imidazolium-bridged dinucleotide intermediate, and the reaction of the intermediate with the primer on the template to produce the primer extension product. Both the intermediate structure and, in the case of the activated auxiliary oligonucleotide, the additional pre-organization of the RNA duplex structure, affect the rate at which the primer extension process proceeds. The role of the catalytic metal ion in the reaction pathway is currently poorly understood [15].

To better understand the primer extension process, Zhang et al. co-crystallized stable phosphonate analogs with a primer-template duplex [16]. The initial theory proposed that the 3'-hydroxyl group of the primer would react with the incoming phosphate of the activating monomer, altering the leaving group and causing the primer to expand into a nucleotide. They found that when two activated nucleotides interacted, a 5'-5'-imidazolium bridging dinucleotide intermediate was formed. These findings provided evidence for the feasibility of using 2-methylimidazole-activated nucleotides for non-enzymatic primer extension, as demonstrated by Zhang and colleagues. Structural studies of template-bound GpppG (P1, P3-diguanosine-5-triphosphate) have provided evidence in favor of the hypothesis that the imidazolium-bridged intermediate securely binds the template by two Watson-Crick base pairs. In addition, the bound complex is better prepared for in-line nucleophilic assault by the primer 3'-hydroxyl group due to the conformational restriction imposed by the covalent internucleotide bridge [17]. Szostak's group used time-resolved crystallography to identify RNA primer extension that does not require an enzyme. In order to experimentally explain the mechanism of non-enzymatic primer extension, they discovered that the activated ribonucleotide connected to the template produces an imidazolium-bridged dinucleotide intermediate, which may then build a new phosphodiester bond between the primer and nucleotides [18].

Daniel Duzdevich et al. used deep sequencing to investigate RNA primer extension without enzymes. Their approach, NERPE (non-enzymatic RNA primer extension; see Fig. 1), is predicated on a stencil-directed RNA replication technique. To detect mismatches, examine for non-enzymatic ligation, and precisely quantify primer extensions on preset templates, they used a technique they called NERPE-Seq. However, when the single-stranded RNA in the handle is covered by a complementary strand to generate a duplex RNA, their research shows that the primer can successfully extend to the template [19].

DOI: 10.54254/2753-8818/21/20230843

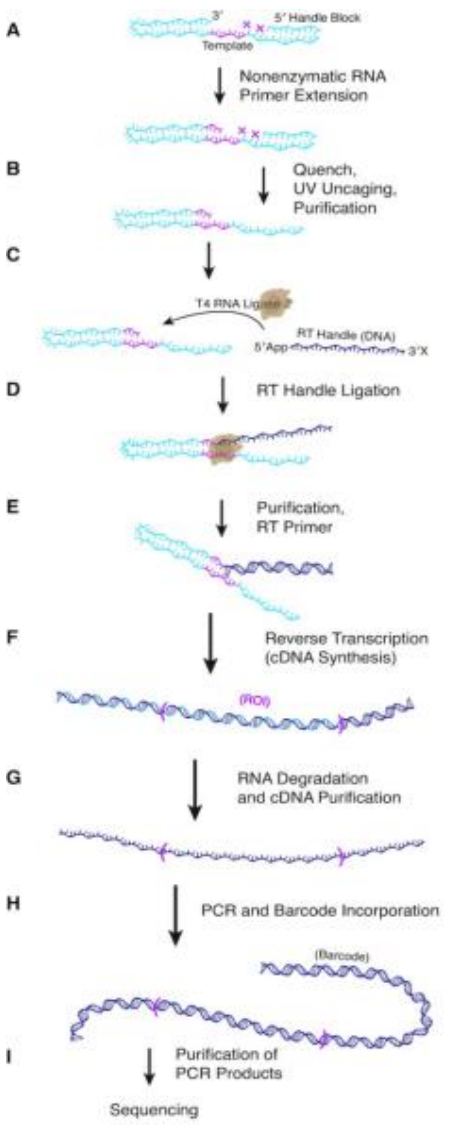

Figure 1. Protocol for preparing RNA hairpin constructs for sequencing:

(A) NERPE-Seq RNA hairpin constructs consist of a hairpin loop connecting the primer and template. This arrangement ensures that the product of non-enzymatic primer extension and the corresponding template are present on a single continuous RNA strand. Two caged bases (represented by magenta Xs) prevent primer extension from encroaching on the downstream 5′ Handle. The 5′ Handle Block, which is complementary to the 5′ Handle, prevents any interference with primer extension.

(B) To quench the primer extension reaction, a desalting size-exclusion spin column is used. The caged bases are then uncaged, and the target RNA is further purified through gel purification.

(C and D) The 3′ end of the RNA hairpin (the site of primer extension) is ligated with the pre-adenylated DNA RT Handle (which is blocked on its 3′ end to prevent self-ligation).

(E) The ligase is removed by digesting it with Proteinase K. The resulting target RNA-DNA complex is extracted using phenol-chloroform. Subsequently, the RT primer is annealed to the RT Handle.

(F and G) Reverse transcription (RT) generates the complementary DNA (cDNA), while degrading the original RNA. The cDNA is isolated using a spin column. The region of interest (ROI) contains the template, hairpin, and any product sequences.

DOI: 10.54254/2753-8818/21/20230843

(H) Polymerase chain reaction (PCR) is employed to add barcodes to the DNA and incorporate flanking sequences. Each barcode uniquely identifies DNA from a specific experiment, allowing the sequencing of samples from multiple experiments simultaneously.

(I) The target PCR products are purified and validated through automated electrophoresis and quantitative PCR before undergoing sequencing [19].

Tracey A. Lincoln and Gerald F. Joyce developed a non-biological system to demonstrate the self-sustaining replication of an RNA enzyme. They conducted two serial transfer studies and found that different reaction conditions and mixtures of enzymes and substrates can produce different outcomes, which may be linked to the nature of the underlying genetic system. They also improved the catalytic properties of the cross-replicating ribozyme [20]. However, the absence of a chemical system that is protocell-compatible and can replicate RNA templates that has all four nucleotides has limited the confirmation of non-enzymatic RNA replication. To bridge the gap between the RNA and non-enzymatic polymerization worlds, the researchers immobilized primers and templates on magnetic beads, added individual nucleotides in sequence, and used short 5' activated oligonucleotides as catalysts to speed up primer extension. By doing so, they successfully created a significant portion of the active hammerhead ribozyme [21].

4. Chimeric phosphoramidate species for RNA copying

Jash et al. discovered that phosphoramidate bonds between the 5'-terminal phosphate of a ribonucleotide and N-terminus of a peptide can form after the interaction between the ribonucleotides and amino in an aqueous condensation buffer. This suggests that spontaneous combination of amino acids and nucleotides can create peptide-RNA hybrid compounds, leading to a peptide-based transfer RNA (tRNA) where the C terminus of the peptide ester-links to the 2',3'- terminus of the oligodeoxynucleotide. The researchers studied how short peptidase RNA interacts with an oligodeoxynucleotide modeled after an RNA strand at its 3′ end. They found that the sequence of the peptide, the dinucleotide's 5'-terminal nucleotides, and the RNA template all affect the speed and efficacy of C-terminal dipeptidyl dinucleotide anchoring to the amino group in their model system. Hybridization close to the primer terminus produced the maximum yield of dinucleotides in all tested cases. The most reactive species, GlyPro-AA, had anchoring yields ranging from 8 to 99% depending on the template. When competing for anchoring at the 3'-UUC-5'-template sequence, LeuLeu-AA, PhePhe-AA, and GlyGly-AA produced a product ratio of 1:2:6, which was independent of the primer's terminal base. These findings suggest that simple double-stranded contexts can control the covalent anchoring of peptidyl RNA at recognized sites of peptidyl tRNA. This process could potentially connect non-template condensation reactions and specialized ribosomal protein synthesis processes [22].

Peptididoyl RNA is the product of ribosome-free single-nucleotide translation and is intriguing from both synthetic and bioorganic perspectives due to its peptide backbone. To stabilize this form of RNA, an amide bond was developed between the C-terminus of a peptide and a 3'-amino-2',3'-dideoxynucleotide in the RNA chain. The preferred synthetic method involved coupling the amino-terminal oligonucleotide to a dipeptido dinucleotide in solution phase using an N-Teoc-protected aminonucleoside support. The resulting hairpin peptide-linked RNA segments (5'-UUGGCGAAAGCdC-LeuLeuAA-3') do not fold cooperatively. This approach enables the creation of doubly RNA-linked peptides on a large scale suitable for investigating their structural and biochemical properties. The presented results demonstrate how a combination of solid-phase chain assembly and template-directed peptide coupling in aqueous solution can prepare RNA-peptide hybrid molecules with the peptide embedded in the RNA backbone. This procedure can create peptidoyl RNAs with different sequences and chain lengths, allowing for the investigation of their chemical and structural characteristics and potential contributions to the prebiotic stage of evolution [23].

Radakovic et al. investigated the role of aminoacylated RNA in templated primer extension and ligation. They found that RNA primer aminoacylation considerably enhanced non-enzymatic replication of RNA templates under certain conditions. Their research also showed that additional

DOI: 10.54254/2753-8818/21/20230843

peptide bonds inside the RNA strand might be created by non-enzymatic ligation using other amino acids. New avenues for ribosome catalysis may open up as a result of these outcomes [24].

The same group was able to assemble a functional ribozyme, which suggests that RNA aminoacylation-encoded peptides may have evolved independently. A key component in translation, aminoacylated RNA can be combined with a template-directed assembly method to create chimeric amino acid-RNA polymers. This chimeric polymer maintains the enzymatic function of all its RNA counterparts, including hammerhead enzymes, RNA ligases, and aminoacyl transferase ribozymes. Previous research by the group showed that short aminoacylated RNAs form amino acid bridges with imidazole-activated oligonucleotides in non-enzymatic ligation processes at a significantly faster rate than unmodified RNAs. By using this approach, the group was able to assemble three chimeric ribozymes, demonstrating that successive ligation processes can produce chimeric amino acid-bridging RNA that is long enough to function as catalytic RNA [25].

5. RNA copying inside vesicles.

In simple living systems, RNAs can serve as both catalysts and genetic information carriers. Encapsulating ribozymes within a lipid-rich environment can enhance their activity and facilitate adaptation. The "rich-get-richer" phenomenon leads to improved RNA catalysts, resulting in greater gains. Protocells can benefit from encapsulated contents, allowing for functional evolution rather than passive containment [26]. According to the stochastic corrector model, population selection among compartments promotes the accumulation of more active ribozymes, reducing errors [27]. Studies by Kevin Leu using various backbone structures (DNA, RNA, etc.) and activated nucleotides show that mismatches significantly slow down non-enzymatic RNA, increasing replication accuracy [28].The process of synthesizing aminoacylated RNA involves phosphorylating amino acids and creating phosphorylated esters before mildly acidolyzing to produce aminoacyl esters. Aminoacyl-tRNAs serve as intermediates in the translation of messenger RNAs into genetically coded protein products, essential for ribosomal peptide synthesis. This process depends on the enzymatic aminoacylation of the 2',3'-diol of tRNAs using aminoacyl-adenylates [29].

6. Homochirality of peptide formation leading by aminoacyl-RNA

The findings of Tamura et. al. suggest that the homochirality of amino acids in proteins may have been produced during aminoacylation and that the RNA may have had a significant role in determining the selectivity (L or D). Prebiotic amino acids that served as asymmetric catalysts for the synthesis of chiral sugars may have also had an impact on RNA chirality [30]. After conducting further research, he discovered that the homologous chirality of contemporary polypeptides is created during aminoacylation and is, in turn, determined by the chirality of RNA that existed prior to aminoacylation. He discovered proof that the aminoacyl transfer step includes a rate-determining phase [31].

7. Evolution view of RNA-peptide world

Bokov et al. developed a hierarchical model to explain the evolution of 23S ribosomal RNA. They identified fifty-nine distinct elements within the 23S RNA molecule, indicating that it did not evolve as a single unit, but rather as distinct components that together maintain the integrity of the ribosome. Through their research, they discovered that an early fragment consisting of approximately 110 nucleotides, which could bind the CCA-3' end of tRNA, was the point at which the evolution of 23S rRNA began. This fragment was replicated and resulted in a molecule capable of binding two CCA-3' termini simultaneously. To enable the transpeptidase process, the two fragments were positioned adjacent to each other in space, allowing the two CCA-3' ends to connect. The ability of these dimers to create oligopeptides with random amino acid sequences likely facilitated their function. In the early stages of evolution, ribosomes were likely little more than RNA molecules. However, the model developed by Bokov et al. does not provide evidence of a complete RNA ribosome [32].

In the early stages of evolution, a steady supply of peptides was essential for the polymerization of activated nucleotides on clay substrates to create primitive RNA molecules. Amino acids could easily

DOI: 10.54254/2753-8818/21/20230843

polymerize to form peptide molecules on the mineral surface, making RNA more stable and easier to produce in the natural environment. RNA and peptides interacted and worked together to broaden their range of function. The current understanding of RNA binding proteins (RBPs) is rapidly expanding, with topics covering techniques for finding RNA binding sites, RBP synthetic design, and the function of RBPs in stress granules and neurodegenerative disorders. A thorough mechanistic comprehension of protein-RNA target interactions is beneficial for all these fields. Intricate molecular structures provide an explanation for RBP's function and binding behavior like the helicase domains preferring to interact with the RNA backbone or particular residues in a YTH m6A reader protein "locking in" a methylated base. The examination of hydrogen bonds that various RNA binding domains create with RNA supports the structural roles of certain domains and illuminates how some domains differ from one another [33]. Peptides played a critical role in the peptide or RNA world hypothesis by reducing the activation energy of chemical reactions, which sped them up. The need for specialized and diverse protein enzymes became crucial for biogenesis in the peptide/RNA world hypothesis. Perhaps the first imperfect translation device was a protein called aminoacylated ribozyme. A key element in the process of translating is the assignment enzymes, like pre-aaRS (aminoacyl-tRNA synthetase), which have changed to bind a particular amino acid to a pre-tRNA molecule. The multilingual enzyme aaRS catalyzes the activation process and connects it with the correct amino acid, according to more studies on tRNA and aaRS. An aminoacyl adenylate is initially produced using an amino acid and ATP. An aminoacyl-tRNA, also known as a charged tRNA, is created when the aminoacyl group is transferred to a particular tRNA molecule. Individual amino acids may be distinguished by the aaRS, which can then activate them with ATP to create their conjugate with AMP [34].

The earliest signs of the proteins transforming to peptide occurred with the creation of the "bridge peptide," which aided the aminoacylation of RNA with particular amino acids. This short peptide binds to a particular RNA molecule and a specific amino acid through stereochemical interactions, encouraging aminoacylation and benefiting the structure of artificial peptides. The hybridization of short aminoacylated RNAs gave rise to bridge peptides, which could then promote interactions between particular RNAs and specific amino acids. Kunnev and Gospodinov suggest that the stabilizing effect of bridge peptides on RNA-peptide complexes would have emerged around the same time as the evolution of the genomic code that is standard. As a result of this RNA-peptide world, ribosomes, ribozymes, and enzyme-directed RNA duplication could have co-evolved during similar era, without the need for RNA-only self-sustaining steps [35]. The hypothesized evolutionary pathway from peptide all the way to aaRS represents an increase in functional complexity and adheres to the continuity rule [34].

The aaRS protozymes suggest that the activation of amino acids catalyzed by peptides originated from a single ancestral gene, allowing the formation of a two-letter coding alphabet in the distant past. This challenges the RNA world hypothesis, which suggests that RNA catalysts came first. The authors propose that the origin of life occurred in an environment where peptides and RNA coexisted and supported each other's early molecular self-organization [36].

The genetic code, which links amino acids to nucleotide triplet codons, serves as the bridge between prebiotic chemistry and biology, as opposed to replication, due to several reasons. Firstly, aaRS enzymes must interact with each other. Secondly, reflexivity intrinsic to the production dynamics of polypeptide aaRS promotes bootstrapping. Thirdly, enzyme seizure of aminoacylation that are RNA-catalyzed will inevitably lead to degraded specificity. Finally, the emergence of the Central Dogma is most likely when duplication and interpretation level of mistakes remain analogous. Catalytic RNA overlooks both the computational nature of translation and the need for catalysts to not only accelerate but also optimize accuracy, leading to an overestimation of the potential catalytic proficiency of ribozymes. Therefore, synchronized evolution of genetic coding was necessary. Protein folding permanently alters genetic information. The Central Dogma guarantees that biological evolution in any RNA world surpasses the simple dynamics of population of natural selection. It is crucial to find experimental evidence supporting the RNA world hypothesis. As the precursors of the first enzymes, aaRS possess three unique functions that grant them special status. Firstly, they

DOI: 10.54254/2753-8818/21/20230843

irreversibly synthesize aminoacyl 5'-AMP by accelerating amino acid activation by a factor of 1014 at the cost of two ATP phosphates. Therefore, the rate of production of prebiotic protein is restricted by the uncatalyzed rates of other protein synthesis reactions, which are orders of magnitude faster than activation. Furthermore, the adenosine functions as an affinity tag that increases the specificity of coding assignment, especially during editing, by a factor of 1000. Finally, they acylate tRNA, where a particular amino acid is attached to a tRNA molecule via a code-cognate anticodon. Code bootstrapping and optimal gene sequences are made possible by the recursive nature of assignment catalysts based on proteins. The search for the optimal code may be substantially accelerated if the protein aaRS tree-like phylogenies offered the possibility of self-organizing quasi species bifurcations. Long polypeptide secondary structures were shown to be structurally complementary to nucleic acids prior to the discovery of catalytic RNA [37].

In today's aaRSs, the conserved modules can be oriented either clockwise or anticlockwise without affecting the enzyme activity. Aminoacyl-tRNA synthetases (aaRS) catalyze two chemical processes essential for complete genetic code translation. Luis Martinez-Rodriguez et al. described 46-residue peptides that contain the ATP-binding sites of Class I and II synthetases, as well as peptides expressed by a gene engineered by Rosetta to make analogous peptides on opposite strands. Saturation can occur for both wild-type and engineered peptides, and the catalysis of both is susceptible to mutations in the active-site residues. The activities of the two peptides show that there are two possible functional interpretations of the information encoded in a gene. These findings lend credence to the theory that the first known peptide catalysts were responsible for the biosynthetic activation of ATP [38].

Class II aaRS must activate all residues required for Class II activity, while Class I aaRS must activate all residues needed for catalysis by Class I active sites. C.W. Carter Jr. and his colleagues have created a model using only bond angles, standard bond lengths, and dihedral angles in which a polypeptide double helix fits precisely into the minor groove of an RNA double helix with the same helix parameters. The model suggests that the two double-helical structures provide some degree of genetic coding and are mutually catalytic for the assembly of one another from activated precursors in the prebiotic soup [39].Two novel modular levels, protozymes and Urzymes, were associated with parallel losses in catalytic proficiency according to parallel experimental deconstructions of Class I and II synthetases. Bidirectional coding supports the essential unification of the proteome. The bidirectional genetic coding of some of the oldest genes in the proteome severely constrains the probability that any RNA World existed before the origins of coded proteins [40].

8. Conclusions

Based on the aforementioned discussions, these suggestions do not guarantee a successful explanation of the origin of life. However, the widely accepted notion of the RNA-peptide world could be crucial in solving this puzzle. Despite having supporters, the RNA-world theory and the RNA-peptide theory are still lacking crucial experimental evidence. Further research into bridge peptides, aminoacylation ribozymes, RNA-binding proteins, and other related topics could provide additional insights into the mystery of life's genesis.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230844

Application and risk analysis of brain-computer interface

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Abstract. As society progresses and science and technology develop, brain-computer interface has made eye-catching achievements. Brain-computer interface (BCI) is a technology that realizes information exchange and response by establishing a direct connection between the brain and external devices. Brain-computer interface is one of the main research directions of human-computer interaction, which has great development potential and wide application in the future. However, the risks and challenges beckoned by it, on the other hand, cannot be ignored. It is of great significance to analyze its application and risk and at the same time to develop the solutions. Based on the existing literature, this paper summarized the working principle of the brain-computer interface, combed its application in various modern fields, and explained the current challenges it faces, hoping to provide certain references for the further development of the technology. Pursuant to relevant studies, it was found that the brain-computer interface has wide application prospects in medical treatment, the economy, people's livelihood, education, and the military, and currently, the main challenges cover inaccurate transmission, low safety factors, obvious ethical problems, and privacy disclosure risk.

Keywords: brain-computer interface, ethical issues, risks and challenges.

1. Introduction

As science and technology develop, the research on human-computer interaction has been focused on the brain-computer interface (BCI). In proper use, it can significantly facilitate the development of modern society. BCI has wide applications and bright prospects in medical treatment, the economy, people's livelihood, education, military and other fields. However, a heap of risks and challenges emerge at the same time.

With the advance of science and technology and the application of artificial technology, the brain-computer interface as an innovative artificial intelligence rehabilitation technology has been applied to daily life. Brain-computer interface (BCI) is a technology that realizes information exchange and response by establishing a direct connection between the brain and external devices [1], in the field of neural engineering, which is among the active research options. Through the marriage of external devices and the human brain, the brain-computer interface can carry out information exchange and control of devices, which plays a vital role in the future development of human science and technology [2]. In this paper, by reviewing relevant literature, the application progress of the brain-computer interface and the challenges encountered were explained, for the sake of providing a reference for the future development of the brain-computer interface.

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DOI: 10.54254/2753-8818/21/20230844

2. The working principle of brain-computer interface

Brain-computer interface, also known as "brain port" or "brain-computer fusion perception", includes signal input, signal processing, data processing, translation, and operating agreement links, which form a complex system with signal collection and matching functions. It can be said that BCI is a technology that realizes information exchange and response by establishing a direct connection between the brain and external devices [2]. According to the degree of intrusion of the interface into the human brain, the brain-computer interface can be classified as "invasive", "semi-invasive" and "non-invasive" [3], and at present, the main research has been focused on "semi-invasive" and "non-invasive".

2.1. Implantation approaches

Invasive signal acquisition implants signal acquisition devices in the cerebral cortex surgically, so as to obtain high-strength and high-resolution neural signals. This method has the advantages of a high precision rate, high sampling rate, and a high degree of freedom, etc., but it is an invasive operation, which may cause an immune reaction and lead to poor signal collection [4]. In order to avoid brain injury due to electrode implantation, at present, most BCI systems use non-invasive methods to collect electroencephalograms (EEG). The semi-invasive signal acquisition means that signal acquisition equipment is implanted between the cerebral cortex and the scalp, and the quality of signal collected is between non-invasive and invasive, which has lower surgery risk and less immune response [5]. Compared with invasive BCI, non-invasive BCI has the advantages of low cost, low risk, simple operation, and easy use. Meanwhile, users' acceptance of non-invasive BCI is much higher than that of invasive BCI [6-7].

2.2. Equipment control

Equipment control involves decoding the brain's electrical activity commands through signal processing and using it to control different devices, so as to induce functional recovery in patients. According to different functions, control devices can be divided into three categories: "function assistance", "function recovery" and "function enhancement" [8]. Among them, the first mainly helps patients to control and communicate with the outside world. Functional recovery mainly helps patients regain lost function; Function enhancement is primarily used to realize the expansion and strengthening of function [8], which is not only limited to rehabilitation medicine, but also has broad application prospects in military, education, and aerospace fields.

3. The application prospect and field of brain-computer interface

3.1. Medical treatment

Currently, rehabilitation instrument commonly used in clinic covers upper and lower limb joint rehabilitation machine, rehabilitation movement machine, etc. Although some facilities for medical use can make the limb function of patients restored to some extent, the issues such as slow recovery and not ideal rehabilitation effects still widely appear in patients. As science and technology advances and artificial intelligence emerges, intelligent rehabilitation technology that directly interferes with the central nervous system has been developed and applied in clinical rehabilitation, among which brain-computer interface technology is the representative. The brain-computer interface directly acts on the brain and can effectively improve the brain nerve conduction pathway [5], so as to help the patients engage in everyday interactions without relying on the nervous system other than the brain and the patient's own muscles. Brain-computer interface technology can greatly improve a patient's life experience during rehabilitation.

In addition, the brain-computer interface has significant effects on the psychological recovery of patients treated. Taking stroke patients as an example, the disease will affect the psychological state of patients, so that they will resist rehabilitation training, and have an avoidant psychology. As you know, the patient's psychological state and participation will have a direct impact on the rehabilitation effect. Brain-computer interface enables patients to simulate physical movements under healthy conditions

DOI: 10.54254/2753-8818/21/20230844

through virtual technology, thereby slowly eliminating their negative emotions and speeding up the recovery process [9]. Studies have found that individual patients only have minor adverse reactions such as brain fatigue and head discomfort during brain-computer interface rehabilitation training, which can be significantly relieved after rest [10-11]. It can be seen that the security of the brain-computer interface is better.

In late March, at the Neuro-disease Branch of the Center for Clinical Translation Research on Brain-Computer Regulation at Zhejiang University, the research team is working hard to decipher brain activity related to mind writing, and the latest research results will be published soon. At present, the research team is conducting large-scale clinical trials, and dozens of clinical patients from all over the country is receiving closed-loop nerve stimulation machine implantation, for the sake of evaluating the long-term efficacy [12].

3.2. Economy

Brain-computer interface can connect the human brain with many working devices, and achieve the goal of fast operating equipment by reading human electroencephalogram, which provides convenience for workers to operate and improves the efficiency of production. What is more, with the help of this technology, bedridden patients and disabled people with mobility difficulties can also return to work, contributing to the construction of a better society and social and economic development. At the World Artificial Intelligence Conference in 2022, the brain-computer interface and its related products such as intelligent bionic hands attracted many visitors to the exhibition. "Brain-computer interface is a technology that connects a person's brain through a computer, detects very weak signals in the brain and directly controls external devices through the computer. In the field of brain-computer interface, there are two main types of technology implementation paths: invasive and non-invasive. "In terms of commercialization, invasive methods mainly solve problems related to severe brain diseases, while non-invasive one has a wider range of commercial application and problem-solving", said Han Bicheng, founder and CEO of BrainCo. According to the "Brain-Computer Interface Technology Innovation and Industrial Development Research Report (2021)" released by the China Academy of Information and Communication Technology, it is predicted that brain-computer interface-related market size may exceed 3 billion US dollars in 2027. The future industry represented by brain science and brain-like research is also becoming a new track for local economic development [13].

3.3. People's livelihood

Brain-computer interface also has promising applications in the current hot field of the Internet of Things, combined with the area, which can enable an intelligent lifestyle and improve people's lives. For example, with respect to smart homes, the current smart home still needs people to take the initiative to make instructions. With the brain-computer interface, home devices can be controlled using only the brain. Likewise, in the aspect of entertainment, brain-computer interface technology can also realize the integration of virtual reality technology, and thus bring users a more convenient and real experience. Beyond that, in 2011, Japanese technology company Neurowear developed brain-computer interface devices called "electroencephalogram cat ears" and "electroencephalogram cat tails". In 2019, American technology company BrainCo developed a mind-enabling head ring that can detect and improve attention and in 2021, Beijing Naolu Technology Co., LTD launched a sleep product Sleep Up.

4. Risks addressed by brain-computer interface

4.1. User security problems due to immature technology

At present, the brain-computer interface is not very mature, which may lead to data loss in the process of information transmission, and the reading of users' electroencephalograms may not be very accurate, which cannot fully reflect users' thinking. The speed of information processing is also far from the almost instantaneous processing speed of humans themselves. Invasive brain-computer interfaces may cause serious problems such as infection during surgery, which may harm the physical health of patients.

DOI: 10.54254/2753-8818/21/20230844

That is to say, due to the limitations of its current technological development, brain-computer interface technology may cause direct harm to users, especially in the links requiring surgical intervention [14]. The reception of electroencephalogram by the brain-computer interface may lead to the reception and decoding of a part of users' mental activity that they do not want anyone to know about. Such excessive use may result in users' personal privacy leaks, and lead to inconveniences and inferiority in users' psychology and life.

4.2. Ethical problems caused by the misuse of technology

From the perspective of modernization, the level of ethical quality is often an important factor affecting the development and progress of technology [15]. Technology development depends on the cultural and social background in which ethics locates, and is affected profoundly by it. Among them, public opinion related to technology is one of the most important factors [16]. The universally recognized and respected values of human society, such as fairness, justice, and diligence, should not be degraded with the innovation and use of new technologies. If brain-computer interface technology gradually penetrates into every field and level of society and forms alienation of the beautiful value of human society by virtue of its excellent "enhancement" effect, and at this time, the public opinion still treats the value impact of BCI with a numb and indulgent attitude, basically we can judge that the development of BCI technology is in a "problematic" cultural and social background, which is extremely unfavorable to the construction and development of this kind of technology. For example, some students, enhanced by the brain-computer interface, have obtained excellent learning abilities that ordinary people can hardly match no matter how hard they try, while the public opinion environment at this time is still numb and inclined to use the final result (rather than the process of hard learning) to judge the value, then the value of diligence, effort and fairness in the field of education will become increasingly weak because BCI users will become more and more arrogant and lazy considering that they can easily make achievements that are beyond the reach of ordinary people without putting in as much effort as ordinary people do; For another thing, those who do not receive BCI will lose confidence and motivation, and will no longer believe that their diligence and effort will make a difference because anyway, they will not be able to reach the heights that "enhanced people" can achieve.

5. Conclusion

In this paper, the pros and cons of brain-computer interface (BCI) as an emerging technology are analyzed by summarizing the articles on BCI and human enhancement technology in recent years. Brain-computer interface, while being expected to help treat a series of serious diseases, enhance human abilities, and improve people's lives, also beckons a series of social, ethical, and safety issues that need to be considered. Despite no small disadvantages in these three aspects, in the face of the huge benefits of brain-computer interface technology, it is worth solving these problems. Moreover, in attempting to apply this technology, we must fully appreciate the complexity of human life and its unique importance, and ensure that this application does not undermine the basic forms of human life and the associated human values.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230845

Artificial intelligence in healthcare: Opportunities and challenges

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Abstract. The development of Artificial Intelligence (AI) in healthcare has had a significant impact on healthcare. AI in healthcare can provide more accurate diagnoses and interventions for patients. AI can predict, diagnose, and treat diseases, facilitate the maximum use of healthcare resources by integrating medical information, increase efficiency, and reduce overcrowding of healthcare resources. However, the application of AI in healthcare also faces challenges such as accountability, algorithmic security, and data privacy. This paper discusses the application of AI in healthcare and explores the challenges faced by AI, including accountability traceability, algorithmic safety, data security, and ethical issues, and makes targeted recommendations. This study provides an in-depth exploration of the application of AI in healthcare, helping to improve the accuracy and efficiency of AI applications in healthcare, as well as providing necessary guidance and references for optimizing and enhancing AI technologies.

Keywords: AI, healthcare, opportunities, and challenges.

1. Introduction

The introduction of artificial intelligence (AI) has had a substantial impact on the healthcare industry. Topol et al. state that AI can facilitate clinical workflow by fostering healthcare data collection and medical information organization [1]. This suggests that AI applications in healthcare can help improve medical efficacy, resulting in AI's growing influence in the healthcare industry, particularly considering numerous global health issues. As life expectancy increases and fertility rates decline in the 21st century, the proportion of the geriatric population continues to rise. By the end of the century, more than 30 percent of the European Union's population will be 65 or older [2]. Biologically speaking, cellular and molecular injury accumulation causes human aging [3]. This implies that as people age, their health deteriorates progressively, with common symptoms including hearing loss, osteoarthritis, diabetes, and dementia [4]. Nearly 95% of the elderly suffer from at least one chronic disease, and 80% suffer from two or more chronic diseases. Chronic diseases can reduce patients' independence, requiring elderly patients to rely on long-term care from institutions or families to perform daily tasks. According to the World Health Organization statistics, by 2030, more than one-sixth of the world's population will be over 60 [4]. Many vulnerable elderly individuals in poor health will place an immense strain on limited medical resources. In 2013, the European Union had a 1,6 million healthcare employee shortage. This shortage is anticipated to reach 4,1 million by 2030 based on a pattern of continuous expansion [5]. The accelerated aging of the population will increase the severity of the shortage of medical personnel. The application of AI in the medical and healthcare disciplines can maximize medical resources and alleviate

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DOI: 10.54254/2753-8818/21/20230845

a situation where medical resources are in short supply. This will help accomplish one of the sustainable development objectives of the United Nations, which is to ensure that people of all ages live healthful lives [6]. However, using AI tools can result in various medical errors, including algorithm errors that result in life-threatening misdiagnoses and data integration errors that lead to unnecessary treatment [7]. In the absence of explicit legal accountability for medical errors caused by AI, patients, and physicians will be hesitant to implement AI tools in the healthcare industry, particularly due to concerns regarding algorithm security.

2. Applications of AI in healthcare

By incorporating clinical data, AI-based medical applications have the potential to diagnose, predict, and treat diseases [1]. In heart disease, ML algorithms have been used to calculate the 10-year risk of developing cardiovascular disease, resulting in more accurate cardiac risk scores [8]. Moreover, AI-assisted prediction using clinical data records can be more precise than statistically-derived risk models, indicating that incorporating AI into medicine and healthcare could improve patient care by allowing for more accurate diagnoses and interventions [9].

Moreover, by incorporating physiological data from patients with renal diseases, AI tools can predict the incidence of acute kidney injury (AKI) within 48 hours of hospitalization and the post-operative risk of AKI surgery. In addition, the diagnostic accuracy of AI prediction models can be comparable to that of medical personnel, and their efficacy can be enhanced when combined with other tools [10, 11].

Incorporating AI into healthcare can alleviate healthcare congestion in EU nations with insufficient medical personnel. By prioritizing patients based on their medical data and health status, predictive models utilizing AI algorithms can aid medical personnel in developing higher-quality surgical plans, thereby optimizing healthcare resources [12]. AI can also assist in analyzing emergency department patient arrivals, enabling the development of efficient resource allocation strategies [13-15].

In 28 EU countries, mental illness costs more than 4% of the gross domestic product [16]. AI can provide patients with conversational companionship and emotional support without healthcare personnel treating mental illness [17, 18]. Interactive chatbots can digitally monitor patients' emotions using voice and facial recognition sensors, providing patients with the emotional support they require.

The bureaucracy of the healthcare system requires healthcare workers to spend fifty percent of their time on administrative duties, such as patient data acquisition and devouring valuable resources [19]. By performing these duties more efficiently and precisely, AI can save healthcare professionals valuable time and reduce the staffing shortage.

3. Challenge

Utilizing artificial intelligence (AI) in the healthcare industry presents numerous obstacles. As a comparatively new application, its use is not governed by established laws and regulations, which could potentially injure its users. In cases where patients are injured due to medical errors, the lack of traceability in medical AI makes it difficult to assign responsibility. Artificial intelligence (AI) in the diagnosis or treatment process further complicates the relationship between physicians and patients [20], which could reduce their propensity to employ AI.

Concerns over algorithmic security could threaten the viability of AI medical instruments. Unfortunately, in 2020, the AI company Cense AI was the victim of a cyber-attack, which exposed the sensitive information of more than 2.5 million patients worldwide, including personal diagnosis records, private addresses, and names [21]. The risk of data security incidents makes it more challenging to promote medical AI tools because no one wants their private information, such as their name and address, to be extensively distributed.

The ethical considerations of AI use further complicate the healthcare industry's propagation of AI tools. Individual patient data is regarded as a commodity on the market from the perspective of surveillance capitalism, and sales strategies are devised to increase their purchases. In addition, the pharmaceutical industry uses patient data for drug development and marketing strategies, which may raise ethical concerns [22].

DOI: 10.54254/2753-8818/21/20230845

Moreover, data-level errors can impact the precision of AI predictions. During the ultrasound scanning process, for instance, human error can cause the input data of AI tools to be discordant with the patient's actual condition, which can be understood as data noise. Due to operator ineptitude or uncooperative patients, data disturbance may exist [23].

A 2021 survey of 6,000 people revealed that the majority of individuals need more knowledge of AI's use in daily life [24]. This suggests a considerable danger of data disturbance when diagnosing patients from the general population using AI tools.

Even worse, there needs to be more instruction in extant curriculums that seeks to teach clinically trained physicians how to use AI tools. A study conducted at 19 institutions in the United Kingdom revealed that medical students are required to take only a handful of AI courses [25]. Healthcare personnel still need to gain the experience necessary to be the dominant consumers of AI tools, so they cannot guide and assist patients and may generate data noise. A patient donning a wedding ring may position their hand on their chest during the scanning procedure. An X-ray technician may place an adhesive electrocardiogram electrode on the chest. These circular artifacts may be misidentified as one of the known thoracic lesions, resulting in false-positive results [26]. The data pollution caused by these actions will result in algorithmic errors in artificial intelligence and increase the risk of misdiagnosis. The risk of erroneous diagnosis due to improper application will impede the widespread adoption of AI tools in the medical and healthcare sectors.

Moreover, data collected from different hospitals and machine learning models used to differentiate between distinct populations may result in incorrect classification by AI due to changes in the dataset's quality [27]. This implies that the data model will reduce the accuracy of predictions even in the absence of data disturbance. Using optical coherence tomography (OCT), DeepMind has created an AI digital model system that can automatically diagnose retinal diseases. However, the diagnostic error increases from 5.5% to 46% when the AI system obtains data images from multiple devices [28].

As it is typically designed by computer and data scientists, the development of medical AI technology often needs more input from end-users such as patients, nurses, and physicians [29]. This absence of involvement can make it difficult for users to comprehend and employ these tools effectively, increasing the likelihood of human error when using AI tools.

In addition, the development of medical AI tools involves a large number of participants, resulting in a lack of transparency in the development process, which further inhibits the use of AI tools in diagnostic methods by users who do not comprehend how AI models operate in the real world [30]. This lack of transparency makes it difficult to determine who is responsible for possible errors, whether AI developers, data administrators, physicians, or others.

Moreover, the complexity of informed consent procedures and the lack of transparency of AI algorithms may necessitate that patients comprehend how their data is utilized and shared, posing potential ethical risks [31]. Establishing data protection laws to ensure the responsible use of AI tools in healthcare is crucial.

4. Suggestions

To optimize algorithm safety, technical research, laws, regulations, and policy systems, and increased transparency and interpretability of algorithm applications are needed to ensure that AI applications in healthcare can be carried out safely and securely. The application of AI in healthcare also needs to take ethical issues into account. The safe application and promotion of AI in healthcare can be promoted by developing ethical guidelines and codes and increasing education and awareness to serve patients and healthcare organizations better. Ethical issues such as patient privacy and data protection must be considered when using AI. The balance of interests between healthcare providers, doctors, patients, and technology companies needs to be considered when developing guidelines and norms. There is also a need to consider how to ensure the fairness and transparency of algorithms in the development and application of AI.

From the perspective of AI use, the skills and awareness of healthcare professionals should be improved. Healthcare professionals should receive professional training on how to use AI tools properly.

DOI: 10.54254/2753-8818/21/20230845

Manufacturers and providers should provide transparency for AI and inform patients about how their data is used and shared. Governments and hospitals should have laws and policies to protect data when AI is used. This includes specifying what data can be used, how it is collected and used, and how patients' privacy is protected.

5. Conclusion

Artificial intelligence (AI) has made significant contributions to the healthcare industry by improving diagnostic accuracy and relieving pressure on limited healthcare resources. It has improved the comprehension and treatment of various cardiovascular, renal, and psychological diseases. Concerns about data privacy and security have accompanied the advantages of AI in healthcare, as cyber attacks have resulted in the disclosure of sensitive patient information. In addition, data disturbance and shifting can result in the misdiagnosis of patients, resulting in severe consequences and liability issues. To ensure that AI tools positively impact the healthcare industry, it is essential to strengthen system security and protect patients' sensitive data from potential attacks.

In addition, patients must be completely apprised and provide consent before using their data. Although these measures may be costly and time-consuming, the long-term benefits of utilizing medical AI tools outweigh the costs. AI has the potential to enhance patient outcomes and increase the efficacy of the healthcare industry, even though it presents some challenges in healthcare.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230847

Vaccines for prevention of respiratory syncytial virus infection

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Abstract. Respiratory syncytial virus is a virus that causes infections in lower respiratory tract among infants aged under 5 years old, older adults aged above 65 years old and populations with other potential complications. Some susceptible populations to RSV may develop pneumonia and bronchiolitis. The mechanism of RSV virus transmission is through air droplets. The burden of hospitalization causes by the infection of RSV is severe and episodic, with the prevalence mainly concentrated in winter periods. The development of vaccines against RSV virus statutes in the early 1960s, but was paused and halted due to the failure of formalin inactivated vaccine. The current study first reviewed the prevalence, structure, mechanism and development history of RSV and its vaccines, following which the present work focus on a summary of the results of clinical trials of vaccines against RSV on different groups of populations, especially those with special condition, such as older adult and pregnant women.

Keywords: RSV, infants, older adults, pneumonia, bronchiolitis, mechanism, clinical trials, formalin inactivated vaccines.

1. Introduction

1.1. Respiratory syncytial virus and infection

Respiratory Syncytial Virus (RSV) is a pathogen which causes lower respiratory tract infection and associated respiratory illnesses, such as bronchiolitis. When infected with RSV, the majority of the susceptible population of RSV, including children who are aged under 5 years old, elderly aged above 65 years old and the population who possesses other potential diseases, develops clinical symptoms, such as sneezing, cough, fever and etc. On the other hand, the virulence of RSV is less severe for adults compared to the virulence for its susceptible population [1, 2]. Susceptible population of RSV may also develop complications like pneumonia, middle ear infection or asthma later. In addition to the previous complications, RSV is also to be found as a major cause of bronchiolitis in 1957. The prevalence of RSV focuses during winter and it varies among different populations [3]. For instance, the prevalence of RSV among adults is 3-10% in each winter. Similar to influenza, RSV infections among adults are usually more serious and elongated than the common cold. The trend shown by RSV infection indicates that it increases along with age of the adults and other risk factors. For instance, the proportion of hospitalized adults aged above 65 years old among the elderly who are infected with RSV is approximately 1 or 2 per 1000 people. These population develops pneumonia or aggravated potential cardiopulmonary complications, with a case facility rate of 1 to 2%.

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DOI: 10.54254/2753-8818/21/20230847

1.2. Mechanism of RSV infection

The structure of RSV mainly consists of two types of glycoproteins: attachment glycoprotein and fusion glycoprotein, which contribute to the initiation of infection. Attachment glycoprotein infects the ciliated cells in the airways, while fusion glycoprotein causes the surface membrane of the virus to combine with the target cell's membrane. In addition to ciliated epithelial cells, RSV virus also infects $CDT4^+$ and $CDT8^+$ T lymphocytes, which results in the reduction of interleukin 2 and interferon y production [3]. After the virus infection, both attachment and fusion glycoprotein will combine with the neutralizing antibodies and trigger an active immune response and the patients will develop clinical symptoms. RSV infects people with high exposure as a form of droplets produced by coughing and sneezing, which later contacts with the patients' eyes, nose and mouth. Also, the droplets are able to land on inorganic surfaces and the RSV can be transmitted by touching the surfaces, given that the patients do not clean their hands with soap afterwards.

1.3. History and development of RSV vaccines

The earliest development of RSV vaccine can be traced back to the 1960s [4]. Before the initiation of development of RSV vaccine, the whole-inactivated polio vaccine invented by Jonas Salk was proven to be a stable intervention against polio.

It was validated in 1955. Since the 1960s, the cases of paralytic polio in the United States decreased from thousands to a few of dozens each year. After the success of the polio vaccine, researchers started the development of whole-inactivated RSV vaccine. Furthermore, researchers conducted 4 studies with large scale in 1965 and 1966, in which the experiment participants were vaccinated before 6 months of age and before their first RSV infection. Nevertheless, the result of the experiment was disastrous. An RSV outbreak occurred within the youngest experiment participants during the winter of 1966 to 1967. Among the 31 immunized infants, 20 were infected, 16 were transported to hospitals, 2 died [2]. Subsequent investigation revealed the whole-inactivated vaccine developed in the early 1960's did not offer protection against RSV diseases to infants. Moreover, the vaccine aggravated the virulence of the diseases. As a result of the tragedy from 1966 to 1967, the development of RSV vaccine paused.

Despite the high prevalence of RSV infection in young children, there is lack of effective treatment against RSV and the routine management are supportive therapy [5]. Several newly-developed vaccines have been tested for the prevention. An example of protection against RSV is palivizumab, an artificial monoclonal antibody, which is validated for prevention of severe RSV illnesses to infants with potential risk factors [6]. Other monoclonal antibodies are also proven to effectively prevent against RSV fusion protein by clinical trials, such as nirsevimab and motavizumab. On the other hand, there are some improvements currently regarding on the allocation of multiple doses of palivizumab during epidemic, on the suggestion of the antibody use on a small population of infants who are susceptible to severe RSV illnesses, on the effectiveness of the antibodies, which is about 45 to 55% among susceptible infants, and on their prices. Despite these improvements that needed to be implemented in the future, researchers have proven these monoclonal antibodies are relatively reliable.

2. A summary of clinical trials in RSV vaccine

In the following section, we reviewed the clinical trial results regarding vaccines against RSV infection with a focus on infants, older adults and pregnant women.

2.1. The results of clinical trials in infants

Currently, there are some candidate RSV vaccines that can be injected into infants [7]. For example, a randomized, double-blind phase 1 trial was conducted among 114 children aged from 1 to 59 months old, in order to test the efficacy of candidate live-attenuated, intranasal RSV vaccine cpts-248/404 on children. cpts-248/404 was infective between 104 and 105 plague-forming units in RSV-naive children. Also, the vaccine was widely immunizing among children greater than 6 months old. Antibody responses among serum and nasal cavities only showed the activity of IgA antibody and they had prevailing response towards RSV attachment protein, with no increase signs of neutralizing activity.

DOI: 10.54254/2753-8818/21/20230847

However, the release of viral load was limited on the second dose and initial evidence showed that second infection of RSV is able to prevent severe clinical symptoms. Overall, candidate vaccine cpts-248/404 did not lead to lower respiratory tract infection or other related clinical symptoms. Nevertheless, the vaccine has a potential harmful effect among the youngest infants, since upper respiratory tract obstruction may occur during viral recovery with the highest rate. The cpts-248/404 vaccine candidate did not cause fever or lower respiratory tract illness. In the youngest infants, however, cpts-248/404 was unacceptable because of upper respiratory tract congestion associated with peak virus recovery. Therefore, the live attenuated RSV vaccine designed for infants will have additional mutations compared to typical cpts-248/404 candidate vaccine.

Another example is B1 cp-52/2B5 (cp-52) candidate vaccine, which is a live-attenuated vaccine made in the combination with Vero cells [8]. However, the clinical trials on infants indicates that the vaccine was over-attenuated. Later analysis shows the vaccine has a large section of coding sequence of hydrophobic and attachment proteins deleted, which results in the absence of a complete attachment protein. Despite the incompleteness of the viral structure, the virus of the candidate vaccine remains replicable and infectious to stimulate immune response. Cp-52 candidate vaccine shows partial deletion of genes of RSV virus may provide a new approach of developing live attenuated vaccines.

2.2. The results of clinical trials in older adults

Currently, there are some probable approved candidate RSV vaccines that can be used for older adults [9]. For instance, a phase 3, placebo-controlled trial is being conducted among 17 countries in the globe in 2023. The researchers have assigned single doses of AS01-adjuvanted RSV pre-fusion F protein-based uncertified vaccine E or placebo to experiment participants, who are above 60 years of old, in a 1:1 ratio before RSV prevalence. Before the trial starts, all participants have known the agreement and potential risks from written or visual documents. The aim of the trial is investigating the effect of single dose of RSV pre-fusion F protein-based vaccine protecting against RSV-associated lower respiratory tract illnesses during single RSV prevalence. Researchers expect the efficacy of the vaccine that it should remain within 20 percent of correction of the confidence interval. During the clinical trial, researchers assess and analyze the efficacy of the invalidated pre-fusion F protein-based vaccine against serious RSV-associated lower respiratory tract illnesses in accordance to RSV subtypes. Meanwhile, safety measures are also conducted.

Among 24,966 participants of the trial, 12,467 of them is injected with single doses of RSV pre-fusion F3 OA vaccine, while other 12,499 participants are injected with placebo. In the following median of 6.7 months after the end of the study, the vaccine efficacy on protecting against RT-PCR-confirmed RSV-associated lower respiratory tract is 82.6% (the 96.95% confidence interval of the vaccine lies between 57.9% to 94.1%). Among the group which the participants are injected with the vaccine, 7 participants are infected with RSV (equivalent to 1 per 1000 people), while 40 are infected in the placebo group (equivalent to 5.8 per 1000 people). On the other hand, the efficacy of the pre-fusion F3 OA vaccine is 94.1% against serious RSV-associated lower respiratory tract illnesses, with a confidence interval of 95% (confidence lies between 62.4% and 99.9%). Finally, the efficacy of the pre-fusion F3 OA vaccine is 71.7% against RSV-associated acute respiratory tract infection, with a confidence interval of 95% (the confidence lies between 56.2% to 82.3%). Specifically for RSV subtype A, the vaccine has an efficacy of 84.6% for RSV-associated lower respiratory tract illnesses and 71.9% for scute RSV-associated infections. For RSV type B, the vaccine has an efficacy of 80.9 towards RSV-associated lower respiratory tract illnesses and 70.6% towards acute RSV-associated respiratory infections. Overall, the data collected from the trial indicates that the effect of RSV pre-fusion F3 OA vaccine is stronger on type A RSV than type B RSV. Nonetheless, the efficacy of the vaccine is significant among participants with different age groups and with various health risk-factors. The reactogenicity of RSV pre-fusion F3 OA vaccine is stronger than placebo. However, the side effects cases reported during the trial are relatively mild to moderate in the level of severity. The incidence of the difference of severe side effects of the vaccine is similar among the two trial groups [9].

DOI: 10.54254/2753-8818/21/20230847

In conclusion, the RSV pre-fusion F3 OA vaccine is proven to be relatively reliable to elderly above 60 years of age. From this international phase 3, placebo-controlled trial, one dose of the RSV pre-fusion F3 OA vaccine is able to prevent acute RSV-associated lower respiratory infections and illnesses among elderly aged above 60 years old, without the factor of the RSV subtype and the underlying potential health risk factors of the infected patients [9].

However, there are also some unapproved candidate RSV vaccines for older adults [10]. For example, a randomized, double-blind phase 2b study was conducted between 2015 to 2016 to investigate the effectiveness of a pre-fusion RSV vaccine's prevention on acute RSV associated respiratory illnesses. The investigation assessed 120 μg of RSV post-fusion F protein in a combination with 5 μg of glucopyranosyl lipid assistance, in 2% of stable emulsion. Participants aged 60 years old or above were randomly allocated in a ratio of 1:1. One of the groups would receive the candidate vaccine, the other group would receive placebo (inactivated influenza vaccine). After observations, participants who developed clinical illnesses would be recorded and whose blood and nasal swab would be taken for testing.

Among 1894 participants, 1.7% of the vaccine recipients developed acute RSV-associated respiratory illnesses for more than 14 days. For placebo group, 1.6% of the participants developed acute RSV-associated respiratory diseases. The vaccine efficacy was indicated as 7.1%, with a 90% confidence interval between 44.3% to 106.9%. The effect of the vaccine was not observed in the following analysis, which ranges from seroresponse to non-vaccine RSV antigens. Efficacy of the vaccine is 8.9%, with a confidence interval between 28.5% to 35.4%. When clinical symptoms combined with seroresponse, the vaccine efficacy was 10.0%, with a 90% confidence interval between 44.4% to 45.4%. On the 29th day of the investigation, 92.9% of the vaccine recipients generated anti-F immunoglobulin G antibody and established seroresponse. Consequently, 48.5% of the participants receiving vaccines had local systematic solicit symptoms, while 30.9% of the participants receiving placebo had systematic solicit symptoms [10].

In conclusion, although the candidate RSV pre-fusion vaccine showed immunogenicity, its efficacy was below the expected data and did not protect older adults against acute RSV-associated respiratory diseases effectively [10].

2.3. The results of clinical trials in pregnant women

In 2022, some researchers conducted a phase 2b trial, who randomly allocate pregnant women to receive 120 μg or 240 μg of RSV pre-fusion vaccine in addition (or not) of catalyst aluminum hydroxide, or equivalent amount of placebo. When experimenting, the researchers include safety precaution and immunogenicity precaution, which ensures that 50% concentrated of RSV subtype A, B and a combination of both subtypes' neutralizing antibodies are delivered as forms in maternal serum, in umbilical-cord blood and in maternal-to-infant transplecental transfer. The concentration must remain in the required ratio [11].

The scheduled, phase 2b trial contains 406 women and 403 infants. Of all 406 women, 327 are injected with RSV pre-fusion vaccine. The majority of the reactions after vaccination are mild to moderate, while the local reactions are higher among the women who receive the vaccine and aluminum oxide, compare to the women who only receive the vaccine. The percentage of side effects occurring within women and infants is similar among the vaccine and placebo groups, which the events' type and frequency are accordant with the background incidences among the participants. The probability of the variety and frequency of these events were coincident with the background incidences among pregnant women and infants. The geometric mean proportion of 50% neutralizing concentration between the infant participants who receive the vaccine and the infants who receive placebo varies from 9.7 to 11.7 for recipients who receive RSV A neutralizing antibodies and the variation ranges from 13.6 to 16.8 for recipients who receive RSV B neutralizing antibodies. The ratio of transferring neutralizing antibodies which travel through placenta varies from 1.41 to 2.10, of which the data is higher for non-aluminum group than for the group with aluminum. Also, the infants whose mothers are immunized have alike

DOI: 10.54254/2753-8818/21/20230847

concentration of antibodies in umbilical-cord blood and similar transmitting ratio which travels in the transplacental passage across analyzed gestational ages [11].

In conclusion, RSV pre-fusion vaccine causes antibody responses with resultful transplacental transport, in the absence of safety worries [11].

3. Conclusion

In conclusion, the risk for susceptible population infecting the RSV virus and develop clinical diseases is relatively high as usual. Despite the high risk of infection, many researchers have planned and conducted clinical trials on populations with different age groups. Among these clinical trials, the majority of the candidate RSV vaccines meet up with approximate expectation and are able to provide relatively effective immune response. The outcomes of success trials indicate that most candidate RSV vaccines may have the potential for administration and for domestic use. However, the exception of the candidate RSV pre-fusion vaccine trial on older adults between 2015 to 2016 did not protect partial participants against RSV-associated acute respiratory diseases, even though it was immunogenic. The failure of the candidate RSV pre-fusion vaccine on older adults indicates that the efficacy of different types of vaccines against RSV remains as a variable. Overall, the development of vaccine against RSV associated illnesses needs to continue and further tests under regulations are needed.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230852

Research on the contribution of REM sleep to procedural learning

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Abstract. Many studies have confirmed the contribution of sleep to memory consolidation. However, the benefit of rapid eye movement (REM) sleep on memory consolidation remains debatable due to discrepant findings. This paper reviewed the latest human studies that employed complex cognitive procedural learning tasks and new learning techniques, including metacognition stimulation and targeted memory reaction (TMR), to provide evidence on the correlation between REM sleep and memory consolidation of procedural learning. Next, the main hypotheses aiming to explain its underlying neurobiological mechanisms were summarized, with a discussion about the striatum's role, the cholinergic system's activity, and synaptic plasticity during REM sleep. Finally, the paper discussed potential reasons for data inconsistency and several aspects that should be considered in future sleep and memory research.

Keywords: rapid eye movement sleep, memory consolidation, implicit procedural memory, motor learning.

1. Introduction

One of the well-known functions of sleep is the processing and consolidation of new memories, which is necessary for efficient learning. Importantly, sleep is not a unitary process, and different sleep stages are likely to play roles in the consolidation of different types of memories [1-2]. Over the past few decades, non-rapid eye movement (non-REM) sleep has been extensively researched. The important involvement of slow-wave sleep (SWS) and N2 sleep spindles in hippocampal-dependent learning, which needed the consolidation of declarative memory, was strongly supported by consistent findings from a number of studies [3-5]. On the contrary, the benefit of rapid eye movement (REM) sleep on memory consolidation remains debatable due to inconsistent data results. Some of the previous studies provide evidence supporting the dual process hypothesis that REM sleep plays a main role in non-declarative, procedural memory consolidation [6-8]. However, there are several studies indicating unaffected procedural memory consolidation after selective REM sleep deprivation [9-10]. Furthermore, later studies have correlated the consolidation of procedural memories with SWS and N2 sleep spindles [11-12]. In order to clarify the function of REM sleep and the mechanism of procedural memory consolidation during sleep for the implications of efficient motor learning, it is important to explore the causes of the discrepant findings by performing a thorough reflection of previous and recent studies. Therefore, this paper first summarized previous findings about sleep and procedural memory consolidation from the perspective of different types of motor learning tasks; next it reviewed the most recent evidence supporting the function of REM sleep on procedural learning in humans; then it

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DOI: 10.54254/2753-8818/21/20230852

summarized theories to explain the neurobiological mechanisms underlying procedural memory consolidation during REM sleep; and finally it discussed potential explanations for data inconsistencies as well as pointed out aspects that should be considered in future sleep and memory research.

2. Consolidation of various types of procedural learning

Procedural memory, as a type of long-term memory, stores implicit knowledge of skill-learning, often described as "knowing how". The performance of procedural learning can be greatly improved by repetition and practice. Early in the review by Smith, various types of tasks used in sleep studies were summarized (see Table 1) [13]. Simple motor procedural tasks are likely to include non-verbal, fine motor learning without cognitive factors. Those mostly used in the previous research included finger-sequence tapping and pursuit rotor. Another broad category of procedural motor tasks is considered "complex" due to the involvement of a complex cognitive adjustment. Common ones include mirror tracing and Tower of Hanoi. Discrepant findings existed in studies using simple motor tasks. Fischer et al. reported that improvement in the finger-sequence tapping task was positively correlated with the amount of time spent in REM sleep [8]. The same task, however, yielded conflicting findings according to Nishida and Walker: a strong link was identified between the quantity of stage 2 non-REM sleep and performance improvements [12]. With respect to the pursuit rotor task, early in 1994, Smith and NacNeill found that deprivation of stage 2 sleep, rather than REM sleep, significantly inhibited memory processing of the task [14]. Later, Peters et al. found a factor in participants' initial task performance based on the findings that performance improvement was proportional to an increase in stage 2 spindle density in the high-skill group (i.e., participants who showed higher initial performance), while the low-skill group showed a significant correlation between REM density and performance enhancement [15]. The authors thus proposed a model that sleep stages impact memory consolidation of procedural learning depending on the novelty of the motor task and the initial skill level of the individual to explain the different roles of REM and N2 non-REM sleep on simple motor learning. Contrarily, studies using cognitive procedural tasks had more consistent results with regard to the contribution of REM sleep on memory consolidation. For instance, Plihal and Born discovered that following the late sleep retention interval as opposed to the early sleep retention interval, the amount of time needed to complete the mirror trace task was dramatically reduced [2]. Additionally, Smith et al. showed that after completing the Tower of Hanoi and mirror trace tasks, there was an upsurge in both the total number of REM and REM densities [16].. Interestingly, the study also found that participants with higher IQ scores showed a greater increase in the intensity of REM sleep after the training and more improvement in task performance in the retest. This finding, together with the result from Peters et al., provided important insights that the initial skill or intelligence level as an aspect of individual differences should be included in the consideration.

Table 1. Types of Common Procedural/Implicit Tasks in Memory and Sleep Studies.

Name of task	Task characteristics	Type of sleep necessary
Wff logic task	cognitive, non-verbal	REM
Word priming	visual, verbal	REM
Corsi block tapping task	cognitive, visuospatial, non-verbal	REM
Tover of Hanoi	cognitive, non-verbal	REM
Pursuit rotor	fine motor, non-verbal	Stage 2 (N2)

DOI: 10.54254/2753-8818/21/20230852

3. REM and procedural memory consolidation: recent evidence

In the past 10 years, scientists studying procedural memory consolidation during sleep have focused on the use of complex cognitive procedural tasks with the application of some of the latest learning techniques. Brand et al. examined REM sleep's role in transferring implicit procedural knowledge with metacognitive stimulation [17]. Metacognition refers to the ability to regulate one's own learning of procedural knowledge and understand how it can be applied. In order to reduce the influence of subjective factors, the experiment randomly assigned the participants. The Tower of Hanoi cognitive procedural task was taught to both groups, but only the experimental group received metacognitive stimulation. Sleep data were recorded, and re-assessment was conducted the next morning, including a harder version of the Tower of Hanoi task. Participants in the experimental metacognition group had a significant increase in time spent in REM sleep on the post-training night, and showed better performance in the task during re-assessment. The results of this investigation reveal that REM is crucial for the memory processing of procedural knowledge after metacognitive learning. Regrettably, there are no statistics available on sleep variables like REM density.

Suzuki et al. trained participants on a visual discrimination task, which requires implicit procedural knowledge to discriminate the orientation of letters and diagonal bars on the target screen [18]. Sleep variables were recorded on an experimental night, and a retest session was conducted the next morning. Improvement in performance on the task was found to have a positive correlation with EEG alpha power during REM sleep. However, it is worth mentioning that Crupi et al. came to a different conclusion using sleep deprivation [11]. After learning a similar visuomotor task, participants went to sleep. For those in the experimental group, acoustic stimuli were applied to suppress slow wave activities (SWA). As a result, visuomotor performance improved significantly under the control condition, whereas overnight improvement was inhibited under the experimental condition of slow-wave deprivation. The finding led to the conclusion that SWA plays a causal role in procedural memory consolidation using visual motor tasks.

Through the use of targeted memory reactivation (TMB), Picard-Deland et al. more recently examined the impact of various sleep stages on whole-body procedural learning [19]. In light of the previous discovery, it is possible to promote memory consolidation during the sleep by offline resetting memory traces with a conditioned cue. The participants had virtual reality (VR) flight training before going to sleep while being exposed to task-related tones during SWS or REM sleep. The control group did not receive TMR. Results indicate that learning performance was the best in the condition where TMR was applied in REM sleep compared to the other two conditions, leading to the hypothesis that complex skill learning is most benefited by memory reactivation coexisting with procedural memory processing during REM sleep.

4. Neurobiological mechanisms underlying procedural memory consolidation during sleep

Benefiting from the rapid development of neuroscience techniques, it becomes more plausible and easier for scientists to investigate the neural mechanisms underlying memory consolidation of procedural knowledge as well as the change in brain and neuronal activities during REM sleep. Even though a lot of debates and controversies still exist, several hypothetical models were widely studied as more and more evidence was reported. It is well known that the striatum plays an essential role in motor sequence memory consolidation, which induces the hypothesis that the striatum is also involved in sleep-dependent consolidation of motor sequence memory. Barakat et al. reported functional imaging results that demonstrated an increase in striatal activity during increased non-REM sleep spindle activity, which has been correlated with memory consolidation of simple motor procedural learning [20]. However, Albouy et al. found that the increase in striatal activation was also evident when sleep in the night after the training was restricted, and the behavioral data of the sleep deprivation group showed that task performance was maintained rather than improved [21]. Therefore, in the review by Albouy et al., the authors drew several important conclusions: sleep was not required for the striatum-dependent memory trace to be processed, and striatal activity does not seem to promote improvement in procedural learning but rather the maintenance of performance [22]. Striatum has a function to trigger performance

DOI: 10.54254/2753-8818/21/20230852

stabilization during the early phases of consolidation of motor sequence memory via mechanisms that do not rely on sleep.

A study by Rasch et al. emphasized the importance of the cholinergic system in procedural memory consolidation during REM sleep [23]. Participants first slept for 3 hours during the early night, then completed a declarative word-pair learning task and a procedural finger sequence tapping task. Either a placebo or a combination of nicotinic and muscarinic acetylcholine (ACh) receptor antagonists was administered after learning. Participants then took 3 more hours of sleep to undergo late REM stage. A re-assessment was administered the next evening. It was found that both REM sleep time and REM density were significantly reduced by the blockage of combined cholinergic receptors compared to the placebo condition. Importantly, the results of the retest indicated an evident improvement in procedural task performance was made by the placebo group, while no enhancement was found in the treatment condition with cholinergic receptor blockade. However, no significant difference in performance on the declarative memory task was found between the two conditions. Furthermore, two additional pieces of information the authors provided from the supplemental experiments help elucidate the story. First, serotonergic or noradrenergic substances can inhibit REM sleep as well, but they do not impact memory consolidation for procedural learning. Second, impaired offline consolidation of procedural memory caused by the blockage of combined cholinergic receptors occurs specifically during the late retention sleep but not during a retention interval with wakefulness. These findings specify the involvement of high cholinergic activity during late REM sleep in the consolidation of motor learning.

By monitoring and manipulating neural circuits in rodent models, additional research has recently sought to elucidate the cellular and molecular principles underpinning the function of REM sleep. After learning a motor task, Li et al. discovered that pyramidal neurons' postsynaptic dendritic spines were eliminated during REM sleep in the rat motor cortex, which further facilitated the growth of new spines [24]. The authors additionally demonstrated that REM sleep regulates NMDA receptor-dependent dendritic calcium spikes, which prunes and strengthens newly generated spines. With the approach of sleep deprivation, Zhou et al. obtained the same result: REM sleep deprivation inhibited dendritic spine elimination [25]. It is known that synaptic refinement is involved in experience-dependent learning and development. These studies thus offer potential support for REM sleep's function in the consolidation of procedural learning.

5. Conclusion

In sum, there are several aspects that should be considered before drawing a general conclusion about the relationship between REM sleep and procedural memory consolidation. First, procedural tasks are essentially distinct from each other considering the skills required to complete the tasks. For example, one main characteristic used for categorization is whether the task requires a complex cognitive adjustment. Therefore, questions regarding whether implicit procedural memory can be further categorized into different types with different underlying mechanisms of consolidation occurring at different sleep stages remain unanswered. Second, a number of animal studies provided evidence for the existence of “REM windows”, suggesting that certain periods of REM sleep play a critical role in post-training memory consolidation while times outside of the windows are not necessary for consolidation to occur. It was also suggested that “REM windows” exist in human sleep as well, based on the finding that the fourth and fifth REM periods have a remarkable increase in REM density after learning. Thus, the microstructure of REM sleep should be considered, especially, for studies using sleep deprivation. Third, findings from studies using animal models advanced our knowledge about synaptic plasticity during REM sleep, but more research is needed to confirm its causal relationship with the contribution of REM sleep to procedural memory consolidation. Animal models allow scientists to manipulate neurons and brain circuits at the cellular level. However, limitations do exist. For example, previous studies have confirmed that, unlike in human sleep, SWS and N2 are not dissociable stages in animal sleep. Those aspects provide insights into explaining the inconsistent findings with regard to sleep stages and procedural memory, as well as point out some directions to be studied in the future.

DOI: 10.54254/2753-8818/21/20230852

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230854

Application of brain-computer interface in rehabilitation medicine

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Abstract. A brain-computer interface (BCI) can realize the communication and control between the human brain and computers or other electrical equipment by electroencephalography. It is a novel kind of human2computer interface. BCIs will be applied in rehabilitation, control, and other fields. This paper introduces the working principle of BCIs. Some key techniques to design BCIs are discussed from these aspects: signal processing, device control methods, and signal feedback. Besides, the application of BCI in rehabilitation medicine is also discussed. Finally, some main problems and future trends are pointed out. The discussions have guiding values to the design and research of BCI.

Keywords: BCI, computer science, electromechanical prosthesis, bioengineering, non-invasive.

1. Introduction

Brain-computer interfaces are technologies that establish entirely new communication and control between the brain and computers or other electronic devices that do not rely on conventional brain information output pathways. Brain-machine interface technology in the field of rehabilitation can be done directly through the manipulation of the output device, through the acquisition of the human brain's electric signal, to realize the function disorder of communication with the outside world, to provide a possible way for improving the quality of the impairment of survival. The brain control interface is a new kind of robot interface technology, is a brain-machine interface(also called brain-computer interface (BCI)) important application and the research direction in the field of robot control. Brain-computer connection is the core module of the BCRI system [1].

This article introduces the concept of the brain-computer interface, describes how the brain-computer interface can be used to compensate for lost function or promote rehabilitation therapy, and discusses the application of the brain-computer interface in motor and sensory recovery [2].

2. How brain-computer interfaces work

BCI are direct connections between the human or animal brain (or cultures of brain cells) and external devices. In the case of a one-way brain-computer interface, the computer either receives commands from the brain or sends signals to the brain (such as video reconstruction), but cannot send and receive signals at the same time. Two-way brain-computer interfaces, which allow two-way information

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DOI: 10.54254/2753-8818/21/20230854

exchange between the brain and external devices, are being integrated with prosthetics to develop electromechanical prosthetics for people with disabilities [3].

According to the different ways of obtaining EEG signals, BCI systems can be divided into non-invasive BCI and invasive BCI. Non-invasive BCI involves measuring electrodes attached to the scalp of the brain to record electrical signals. Invasive BCI involves inserting electrodes into specific areas of the cerebral cortex to obtain the electrical activity signals of a single neuron or a small group of neurons... These two methods have advantages and disadvantages: non-invasive BCI has no damage to the user, but the collected signal is fuzzy, the ophthalmoelectric and myoelectric interference is large, and the signal-to-noise ratio is low. Invasive BC1 requires surgical implantation of electrodes into the brain, and the resulting brain signal has a high signal-to-noise ratio and good localization [4]. However, due to some unsolved social issues and user psychological problems, as well as technical limitations, invasive BC1 is only used for specific patients and occasions, and currently, it is mainly tested on animals.

2.1. Signal processing

For the feature extraction of two wavelet packet decomposition tasks in brain-computer interface (BCI), a novel Wavelet packet Decomposition (WPD) and common spatial decomposition is proposed EEG feature extraction method is combined with pattern and CSP. This method first chooses to lead seven important electricals (electroencephalograph, EEG) signals, with 'hear wavelet base third-order WPD decomposition. Then, the five sub-bands of each lead were reconstructed to obtain the relevant frequency domain information. Finally, the reconstructed signal is extracted by the CSP feature to obtain six – a dimensional feature vector. The combination of CSP and WPD can make full use of the time-frequency characteristics of WPD and effectively avoid the defects of CSP requiring too many input leads and a lack of frequency-domain information. The probabilistic neural network (PNN) was used to classify the data from the 2008 International BCI Competition and the experimental data from our laboratory [5]. The classification accuracy of the two data sources was 92% and 80%, respectively, which was 5% and 20% higher than that of simple CSP feature extraction. The experimental results show that the feature extraction algorithm combined with WPD and CSP can extract obvious features and improve the recognition accuracy of BCI.

2.2. Device control

From the perspective of control science, the brain is the control center of all movement and language functions of the human body and sends instructions to the body through the medium of external nerves. Neuroscience research has found that even if the external nerves and limbs lose their function due to injury, the brain function is still normal, and the command information sent by the brain can be transmitted by electrical signals. The study also found that when people are engaged in some thinking activities or induced by some external stimuli, the EEG signal will show a corresponding and regular pattern of change. Thus, people's wishes expressed by abstract and virtual brain activities can be "represented" by real and physical EEG signals, which serve as a bridge between the human brain and the outside world. The above research results provide a scientific basis and working principle for the research of brain control [7]. Electroencephalogram is the basis for realizing such control. Currently, electroencephalogram mainly used for brain-computer interface is as follows: Cortical potential (SCP), P300 potential, Motor imagery (MI) and Steady-state visual evoked potential (Steady-state visual evoked potential) etc. Both SCP and Event-related(de)synchronization (ER(D)S) are independent of external stimuli. And it's generated, it's generated spontaneously. Event-related potential (P300) and steady-state visual evoked potential are evoked modes [8].

2.3. Signal feedback

To train subjects to adjust their status to generate EEG signals suitable for recognition algorithms, a brain-computer interactive feedback system based on virtual reality feedback was designed. The system combines brain-computer interface technology with virtual reality technology. Firstly, the

DOI: 10.54254/2753-8818/21/20230854

virtual human model is built in 3DMAX, then the designed actions are added to the character model in virtual reality. Finally, the system controls the actions of the character model by calling the database in real-time. The simulation results show that the subjects can compare the feedback movement of the virtual person with their imaginary movement in real-time and adjust themselves in time to achieve the consistency of the imaginary movement and the feedback movement. The research results preliminarily prove the feasibility of the feedback system and provide a good idea for the design of the BCI feedback system [9].

3. Application of brain-computer interface in rehabilitation medicine

Brain-computer interface (BCI) technology provides new neuro-engineering solutions for rehabilitation problems caused by amputation or nerve injury. As a result, neural interface technology is being incorporated into rehabilitation strategies for patient populations. After the onset of stroke, patients in the acute phase of the brain-damaged functional areas can have a partial spontaneous recovery, while patients in the sequel stage of the possibility of spontaneously recovering of the cerebral-damage functional regions significantly reduced the traditional rehabilitation treatment methods are not obvious. Especially in the upper limb motor function and hand function recovery and other aspects can only be promoted through some special rehabilitation treatment means to restore the function of stroke often leads to movement, balance, walking, and another aspect of the patient, seriously affecting the patient's daily life activities BCI technology can be collected brain signal processing into executive instructions, and transmitted to the limb through the spinal cord, peripheral nerve. At the same time, the transformed brain control information is presented to the patient through external devices, and the purpose of controlling brain signals is achieved through repeated training, gradually forming a normal cerebral cortex activation state.

3.1. The application of BCI technology in the rehabilitation of upper limb

Application of BCI technology in rehabilitation of upper limb and hand function of stroke patients. Existing studies have preliminarily confirmed BCI in chronic brain clinical effect of upper limb functional rehabilitation in apoplexy patients and its influence on brain functional plasticity. A study of patients with subacute stroke showed BCI training-induced changes in the electro-sensorimotor spectrum of the brain. Improvements in upper limb function in patients were associated with enhanced internal connections in the ipsilateral hemisphere. Li Mingfen et al. believed that electrical stimulation technology based on BCIs has a significant effect on promoting the recovery of upper limb function in stroke patients, and its therapeutic mechanism may be related to promoting the activation of motor-related brain regions on the affected side. Motor imagination can not only improve the upper limb motor function of elderly stroke patients but also significantly improve the cognitive function of elderly stroke patients and improve the quality of life of patients [10].

3.2. Application of BCI Technology in lower limb functional rehabilitation of stroke patients

Hemiplegia after stroke can cause muscle tone changes, often manifested as increased upper limb flexor muscle tension, lower limb extensor muscle tension, limb movement is not coordinated, and then affect the balance and gait, resulting in patients with limited social participation ability, quality of life seriously decreased, traditional rehabilitation therapy training has a certain therapeutic effect, but there is a single treatment method, boring, low active participation rate of patients shortcomings. In recent years, many rehabilitation equipment which can improve balance and gait function have been developed at home and abroad [9].

3.3. Application of BCI technology in stroke speech rehabilitation

Language is an important communication tool for human beings, and it is the main way of expression for people to communicate. Stroke often leads to patients' speech dysfunction, which leads to their limited ability to participate in society. For those with severe language communication disabilities, BCI is the most appropriate and valuable technology at present. It decodes information from the

DOI: 10.54254/2753-8818/21/20230854

patient's brain and converts it into peripheral control instructions. Thus, peripheral control can replace the patient's expression or action. BCI speller is a typical application in this field. It can help people with speech disorders to realize external communication and control through visual or auditory paradigms.

3.4. Application of BCI technology in rehabilitation of stroke consciousness disorder

Brain diseases such as stroke and cerebration injury often lead to varying degrees of disturbance of consciousness in patients. In severe cases, patients even appear unconscious or vegetated. The other person's name is the standard stimulus and your name is the deviant stimulus. The results showed that P300 potential appeared in 5 minimally conscious patients when they heard their name (P300 is a positive wave occurring about 300ms after the deviated stimulus, which can be divided into P3a and P3b components. The classic P300, also known as P3b4 MCS patients had a more pronounced P300 potential when they heard someone else name. No task-related P300 potential was found in the other 5 patients with MCS and all patients with PVS, indicating that the conscious state of the MCS group was significantly better than that of the PVS group The application of brain-computer interface in consciousness rehabilitation of cerebral apoplexy will achieve certain results [7].

3.5. Application of BCI Technology in stroke psychological rehabilitation

After the occurrence of a stroke, most patients' psychologically adverse state is aggravated. Negative and avoidant attitude towards rehabilitation training. At present, most rehabilitation training based on BCI technology, especially VR technology, has added virtual scene videos related to life and physical activities. It can greatly avoid the conflict of patients in the rehabilitation treatment, and improve the enthusiasm and initiative of patients to participate in the treatment, to improve the rehabilitation effect. In terms of limb rehabilitation, two significant roles of BCIs in rehabilitation are replacement and restoration of lost neurological function. When BCI systems are used to replace lost neurological function, the technology restores the user's ability to interact with and control various environments and activities, including computer-based tasks (word processing, Internet browsing, etc.), environmental control units (light, heat, television, etc), mobility devices (power wheelchair drive, or neuroprosthetic limbs) [10].

4. The outlook and challenges of brain-computer interfaces

In recent years, with the rapid development of computer technology and signal processing technology, with the deepening of basic and clinical research on human brain function and EEG, as well as the continuous recognition and attention to the needs and potential of the disabled, the research of BCI has been rapidly developed. In 1995, there were fewer than 20 research groups. In 2000, there are now nearly 100 research groups around the world. A variety of BCI systems are developed in these laboratories. Different BCIs choose different control signals, adopt different signal processing methods, different conversion algorithms from EEG signal to operation control command, and different realization ways of the command output.

After many years of effort, the research of BCI has achieved a lot of exciting results, but it is undeniable that the research is still in the early stage of development. Currently, the maximum communication rate that can be achieved by BCIs is about 25 bits/minl3 J. Most BCIs are still in the laboratory stage, with most testing conducted in normal people and less testing in people with disabilities. There are still a lot of problems to be solved. The research and development of BCI depend on the development and integration of computer science, neurobiology, mathematics, materials science, psychology, clinical rehabilitation, and other disciplines [10].

On this basis, more in-depth research and exploration should be carried out in the following aspects. They are: to improve the information transmission rate of BCI system, reduce the error rate; to eliminate noise more effectively, obtain clear EEG signal, seek effective signal features, the best feature extraction and conversion algorithm, to improve the degree of automation when users use; to explore a more reasonable learning and training method is designed to enable users to control their

DOI: 10.54254/2753-8818/21/20230854

EEG characteristics in the shortest possible time, to reduce the dependence of BCI on conventional motor and sensory output channels; to enhance the mutual adaptability of users and BCI system; to reduce the number of electrodes, reduce the complexity of the use, enhance the stability and compatibility of the BCI system, to develop discipline specifications, accurately and objectively evaluate the performance of BCI.

The development of BCI should pay attention to individual and diversification, in order to meet the differences of individual users and the needs of the extensive application of BCI. Besides, As for the development of innovative BCI, attention should be paid to solving patients' psychological and social-ethical problems while breaking through technological limitations. With the full understanding and gradual solution of these problems, BCI will eventually walk out of the laboratory and into people's lives [11].

5. Conclusion

In this study, the application and challenges of brain-computer interfaces in rehabilitation medicine were studied through a literature review. Brain-computer interface is widely used in rehabilitation therapy, prosthetics for disabled people, and so on. The challenges are inadequate computer power and incomplete signal acquisition. With the development of science and technology, there will be greater breakthroughs in brain-computer interface technology in the future.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230855

Antibody therapies in cancer and autoimmune disease

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Abstract. Cancer and autoimmune diseases are gradually proven to be "two sides of the same coin". Many cancer patients develop manifestations of autoimmune diseases and rheumatism, especially those receiving immune checkpoint inhibitors. At the same time, patients with autoimmune diseases also have cancer combined, which may be related to chronic inflammation damage to DNA or clinical medication. Antibody therapies, pioneered by monoclonal antibody drugs, are now used extensively in the treatment of cancer and autoimmune diseases. The relationship between cancer and autoimmune diseases is gradually being discovered, and some antibody therapies have therapeutic effects on both types of diseases, perhaps because the two have common targets. In-depth study of the role of various targets in the occurrence and development of the two types of diseases, screen the common targets, and discover the antibody drugs that play an activation or inhibition role against the common targets, so as to achieve the effect of "same treatment for different diseases", which brings hope to patients with "cancer-autoimmune diseases". This paper discusses the relationship between the two types of diseases, summarizes the specific targets and corresponding diseases of some antibody therapies, and analyzes the current status of antibody therapy in the treatment of the two types of diseases, in order to explore the "dual therapy" potential of more antibody therapies in the future, and develop new targets and drugs.

Keywords: Antibody Therapy, Cancer, Autoimmune Disease, Monoclonal Antibody.

1. Introduction

Nowadays, cancer remains one of the principal contributors to patient deaths worldwide. The cancer system includes not only cancerous tumor components, but also non-cancerous components and their metabolites, also known as the tumor microenvironment (TME). The TME promotes immune escape of cancer cells, which ultimately leads to cancer tumor resistance.

Autoimmune diseases (AIDs) are diseases due to the bodies' loss of immune tolerance to their own antigens, producing autoantibodies and leading to inflammation production and tissue damage. AIDs are becoming increasingly common and include more than 100 different clinical entities, such as rheumatoid arthritis, dry syndrome, antiphospholipid syndrome, autoimmune thyroid disease and so on [1].

There are growing evidences that cancer and AIDs are "two sides of the same coin" and that they can occur simultaneously despite their opposite mechanisms of action. It has been found that cancer and AIDs share common targets. While antibody therapies targeting the respective disease targets are increasingly being investigated, antibody therapies targeting shared therapeutic targets remain to be

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DOI: 10.54254/2753-8818/21/20230855

explored. Therefore, this paper explores the association between the two types of diseases and summarizes the relevant antibody therapies in order to recommend some directions for progress.

2. Relationship between autoimmune disease and cancer

2.1. Systemic sclerosis and cancer

Systemic sclerosis (SSc), a systemic AID, causes vascular changes and tissue fibrosis, mainly due to immune irregularities, and cancer remains the number one non-SSc-related cause of death among SSc patients [2]. Carbonell et al. used standardized incidence testing and analyzed the risk and factors for cancer in SSc patients and found that SSc patients had an increased risk of cancer ($p < 0.001$), most commonly breast, lung, hematologic, and colorectal cancers [3]. In a regression study by Partouche et al. , breast cancer was the most common cancer among SSc patients within 5 years of being diagnosed with SSc, and gastrointestinal cancer or lung cancer was the most common cancer 10 years after being diagnosed [4]. The positivity of anti-RNA polymerase III (anti-RNAPIII) antibody and anti-Scl-70 antibody were considered as risk factors for cancer in SSc patients [5, 6], anti-SSSCA1 antibody status may also be used as a cancer biomarker in SSc [7].

2.2. Autoimmune thyroiditis and thyroid cancer

Autoimmune thyroiditis, also known as Hashimoto's thyroiditis (HT), is an organ-specific AID caused by the occurrence of an autoimmune process. In this process, two specific antibodies, anti-TPO and anti-Tg, are produced against two antigens, both of which are produced by the body itself, called thyroid peroxidase (TPO) and thyroglobulin (Tg) [8], although negative antibodies do not exclude the occurrence of HT [9]. The most prevalent endocrine malignancy is thyroid cancer(TC), of which nearly 90% is papillary thyroid cancer (PTC) [10]. In a study by Mao et al. HT was found to be a possible high risk factor for TC [9]. HT-associated B lymphocytes located in the secondary lymphoid capsule of the thyroid gland produce and release autoantibodies from inside, leading to shrinkage and fibrosis of the follicles;This stops the infiltration and spread of tumor cells [11], but also increases the false-positive rate during central lymph node ultrasonography [12], which affects the diagnosis and treatment of TC.

2.3. Inflammatory bowel disease and cancer

Inflammatory bowel disease (IBD) is a progressive, refractory disease. Crohn's disease (CD), ulcerative colitis (UC) and indeterminate enteritis are the three most common IBDs. During IBD disease, the intestinal microbial composition is altered, there is an accumulation of Th17 cells in the intestine and an increase in the associated cytokines [13]. IBD can progress to colitis-associated colorectal cancer (CRC) through a process of “chronic inflammatory response - low grade heterogeneous proliferation-high grade heterogeneous proliferative carcinoma” [14]. In this process, Th17 is activated by bacterial components that enter the host intestinal epithelium and lamina propria, promoting CAC tumorigenesis [15].

3. Antibody therapies

Cell surface receptors, such as interleukin, Fc, programmed death, tumor necrosis factor receptors, and leukocyte differentiation antigen, and protein receptors, such as immunoglobulin and complement protein receptors, regulate cell growth, proliferation, and apoptosis through MAPK, AR, Wnt, CASP and other pathways. These receptors and targets have critical effects on the development of cancer and AIDs, and blocking them from functioning has become a new hope for the treatment of cancer and AIDs. For example, immunotherapy approaches that block the PD-1/PD-L1 signaling pathway are used in the standard treatment of cancer [16].

Antibody (Abs) is a glycoprotein secreted by B cells to recognize and neutralize foreign organisms or antigens in humoral immunity. The monoclonal antibodies (mAbs) designed based on the characteristics and effects of antibodies have high specificity, stability and affinity. The mAbs consist of a heavy chain and a light chain, with the Fab segment recognizing cell surface receptors and free molecular surface

DOI: 10.54254/2753-8818/21/20230855

targets, and the Fc segment recognizing Fc receptors on the surface of cells with the ability to kill. Killer cells include NK cells, macrophages and so on. The killing ability of killer cells is activated to achieve specific targeted killing effects through antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) [17].

Today, antibody-associated therapies are no longer only mAbs, but more and more engineered and modified antibodies are available in various coupled forms, such as antibody-drug conjugates (ADCs), bispecific antibodies (bsAbs), antibody fragments (AFs), Fc fusion proteins and so on. bsAbs have the ability to bind to two different epitopes, playing the role of T cell recruitment, double immune checkpoint blockade and so on. The first bsAbs to be authorized for use is Catumaxomab (CD3×EpCAM) [18]. bsAbs are also used for drug delivery, receptor inhibition and activation [19]. On the basis of mAbs, ADCs are coupled with small molecule drugs to form a class of antibody therapy that has both the tumor targeting property of mAbs and the killing effect of small molecule drugs on cancer. The representative ones are trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd) for HER2 positive breast cancer. Their research and development is based on trastuzumab [20].

4. Antibody therapies in cancer

Specific receptors on cancer cells, such as the human epidermal growth factor receptor family (HER/ErbB), can activate downstream signaling pathways, including the PI3K/AKT signaling cascade, to regulate cell proliferation and differentiation, invasion and migration, and angiogenesis [21]. In this process, PD-L1, which is exposed on the tumor cell surface, binds to PD-1 on the T cell surface, thus resisting the killing effect of T cells [22]. Meanwhile, TME contains important cells of tumor immunity, such as Treg cells [23]. Treg cells are CD4+ T cells, which are responsible for suppressing autoimmunity when normally expressing CD25 [24]. Also, helper T cells become two subsets of Th1 and Th2 under the polarization of IL-12 and IL-4. Th2-related cytokines can antagonize Th1 and weaken cellular immunity. mAbs target cancer cell surface antigens, inhibit related downstream signaling pathways, suppress cancer cell proliferation, and kill cancer cells through various killing effects; or prevent the occurrence of immune escape and enhance the host's autoimmune cell-killing ability.

In Table 1, we summarize some of the commonly used antibody therapies and their antibody classes and corresponding cancer names. Nowadays, based on these drugs, more new antibodies are designed, such as the new anti-HER2 mAb H2Mab-181 [25], H2Mab-19 [26], novel mAb developed against KRAS mutations recognizing the extracellular structural domain of human ASCT2 [27]et al; Bispecific antibodies such as Mosunetuzumab, Glofitamab, Oronextamab and Epcoritamab, which target CD3 and CD20, have also emerged, showing good clinical effects in non-Hodgkin's lymphoma [28]. Combinations between different monoclonal antibodies have also demonstrated increased safety and efficacy, such as Epratuzumab, which targets CD22 and is complementary to the known effects of CD20 antibodies [29], the combination of Utomilumab and Rituximab, designed for tumor necrosis factor receptor superfamily member 4-1BB, has demonstrated positive safety and clinical activity in patients with therapeutically resistant/refractory CD20+ non-Hodgkin's lymphoma [30]. Also, certain monoclonal antibodies target targets that have been found in other diseases that have not been studied, and whether they can play a therapeutic role in these diseases remains to be investigated. For example, OX40 expression was found in triple-negative breast cancer (TNBC), and Ivuxolimab, which targets OX40, may be a new drug for TNBC.

However, antibody therapies for cancers may also have side effects. Mogamulizumab, an anti-CC chemokine receptor 4 antibody, has also been reported to induce mossy reactions in mucosal skin [31]; CD3, CD19 dual-target antibody Blinatumomab shows cytokine release syndrome (CRS) and neurotoxicity [32].

DOI: 10.54254/2753-8818/21/20230855

Table 1. Antibody therapies in cancer.

Specific targets	Cancer Name	Drug Name
CD2	Non-Hodgkin lymphoma (NHL)	Siplizumab
CD3 BCMA	Relapsed/refractory multiple myeloma (RRMM)	Teclistamab
CD3 EpCAM	Hepatoblastoma, Non-muscle-invasive bladder cancer (NMIBC), Peritoneal carcinomatosis (PC)	Catumaxomab
CD3	B-cell lymphoma, NHL, Relapsed/refractory follicular lymphoma(FL)	Mosunetuzumab
CD20	Relapsed/Refractory B-cell non-Hodgkin lymphoma (B-NHL), Relapsed/Refractory Diffuse Large B-cell Lymphoma (DLBCL)	Glofitamab Odronextamab Epcoritamab
CD3 CD19	NHL, B-lymphocytic leukemia, Relapsed/Refractory DLBCL	Blinatumomab
CD19 CD22	B-lymphocytic leukemia, Relapsed/Refractory DLBCL	Inotuzumab
CD19	Relapsed/Refractory DLBCL	loncastuximab tesirine
CD20	Lymphoma, NHL	Rituximab Obinituzumab Ofatumumab Ocrelizumab Tositumomab
	NHL, Chronic lymphocytic leukemia (CLL), FL	Ocaratuzumab
	NHL	Veltuzumab
	NHL, CLL	Ublituximab
	Acute lymphocytic leukemia (ALL), NHL, follicular and DLBCL	Epratuzumab
CD22	Relapsed/Refractory hairy cell leukaemia	Moxetumomab pasudotox
	ALL	Inotuzumab Ozogamicin
CD30	Hodgkin's lymphoma(HL), NHL	Iratumumab
	HL, Systemic Mesenchymal Large Cell Lymphoma(ACLC), Cutaneous T-cell lymphoma(CTCL)	Brentuximab vedotin
CD33	Acute myeloid leukemia (AML)	Gemtuzumab Ozogamicin
CD38	Multiple myeloma(MM), RRMM, Newly diagnosed multiple myeloma(RDMM)	Isatuximab
	MM, RRMM, RDMM, Relapsed plasma cell lymphoma, AML, Mantle cell lymphoma(MCL), FL, DLBCL, Blastic plasmacytoid dendritic cell neoplasm (BPDCN)	Daratumumab
	RRMM	Elotuzumab
CD79b	DLBCL , Relapsed/Refractory FL, NHL	Polatuzumab vedotin
CD80	NHL, Relapsed/refractory FL, B-NHL	Galiximab
CTLA-4	Renal cell carcinoma (RCC), Advanced melanoma, Non-small cell lung cancer (NSCLC), Metastatic melanoma	Ipilimumab

DOI: 10.54254/2753-8818/21/20230855

Table 1. (continued).

IL-6/IL-6R	MM, Triple-negative breast cancer(TNBC)	Atlizumab Tocilizumab
	Descending thoracic aorta aneurysm(dTAA)	Satralizumab
OX40	Hepatocellular carcinoma(HCC), Melanoma	Ivuxolimab
4-1BB	NSCLC, Small cell lung cancer(SCLC), Anaplastic thyroid carcinoma, Renal cell carcinoma, Squamous cell carcinoma of the head and neck (HNSCC), NHL , DLBCL, Breast Cancer(BC), FL	Utomilumab
	Urothelial Carcinoma, Bladder cancer(BCa), B-Cell Malignancies, Leukemia, Pancreatic Cancer, Colorectal Cancer(CRC), Head and neck cancer(HNSC), Solid tumors, B-Cell NHL, MM	Urelumab
CC chemokine receptor 4	T-cell lymphomas, T-cell leukemia, Mycosis fungoides (MF) , Sézary syndrome (SS)	Mogamulizumab
EGFR	CRC, BCa, Glioma	Cetuximab Panitumumab
	CRC, BCa, Glioma, Liver cancer	Bevacizumab
	HNSC	Akalux
EGFR MET	NSCLC	Amivantamab
HER2	BC, Stomach cancer	Trastuzumab Pertuzumab
	Metastatic BC	Trastuzumab Emtansine
	Metastatic HER2-positive BC	Trastuzumab Deruxtecan
	HER2-positive BC, Gastric cancer, Gastroesophageal junction cancer, HER2 low expression breast cancer, NSCLC	Enhertu
VEGF	CRC	Bevacizumab
VEGFR2	Stomach cancer, Liver cancer	Ramucirumab
GD2	Neuroblastoma	Dinutuximab
PD-1	Melanoma, NSCLC	Pembrolizumab Nivolumab
	RRMM, Melanoma, NSCLC, Colorectal cancer(CRC), Non-muscle invasive bladder cancer(NMIBC)	Cetrelimab
PD-L1	TNBC, BCa	Atezolizumab
	BCa	Durvalumab
	SCLC, NSCLC, Esophageal squamous cell carcinoma, BC, HCC, TNBC	Adebrelimab
	Soft tissue sarcomas, Biliary tract cancer	Envafolimab
CTLA-4	Metastatic melanoma	Lpilimumab
TROP-2	TNBC	Sacituzumab Govitecan
BCMA	RRMM	Belantamab Mafodotin
TF	Relapsed/Metastatic cervical cancer	tisotumab vedotin-tftv

DOI: 10.54254/2753-8818/21/20230855

5. Antibody therapies in autoimmune disease

In contrast to cancer, most patients with AIDs have Treg cells in peripheral blood that are defective in number and/or function [24]. In many inflammatory and AIDs, CD4+ T helper (Th) cells are involved in tissue destruction [33],Th1-related inflammatory factors have been shown to be relevant with AIDs, for example, Type I Interferons (IFN) [34]. In addition, Th17 cells, which can produce IL-17, have a vital effect on AIDs, and IL-23 on their surface promotes the pathogenicity of Th17 cells in vivo by increasing the production of IL-17 and GM-CSF in RORγt-, STAT3- [35].

Conventional therapies for AIDs are usually glucocorticoids, immunosuppressants and so on. With the emergence of side effects and the development of mAbs, mAbs are also considered as treatments for AIDs. In Table 2, we summarize some information about antibody therapies that can be used to treat AIDs. Comparing Table 1, it can be seen that a variety of mAbs can treat not only oncological cancers but also AIDs, perhaps due to the overexpression of the same targets that can cause both cancer and AIDs. Thus mAbs for the same target may be able to achieve the effect of "treating the same disease",for example, MAP4K3 (also known as GLK) can activate PKCθ in T cells by phosphorylating PKCθ Ser-538 residues, thereby activating IKK/NF-κB, and can also be involved in cell proliferation through the mTOR signaling pathway, and overexpression of GLK can cause cancer, AIDs [36], antibody therapies targeting MAP4K3 (GLK) may offer a new way forward for patients with "cancer-AIDs". It is also worthwhile to investigate whether the existing targets and their corresponding mAbs have a "dual therapy" role in treating both types of diseases.

However, there are still some drawbacks to mAbs for AIDs, such as Alemtuzumab which is thought to be associated with the emergence of secondary AIDs [37]; anti-tumor necrosis factor (TNF) therapy is also not recommended in idiopathic inflammatory myopathy (IIM) because of the potential to induce systemic AID [38]. Even when different mAbs are designed for the same target, they have different efficacy and safety profiles, such as in psoriasis where Risankizumab has been found to have higher efficacy and lower risk [39].

Table 2. Antibody therapies in AIDs.

Specific targets	AIDs Name	Drug Name
CD19	Optic neuromyelitis optica spectrum disorder (NMOSD) , Multiple sclerosis(MS)	Inebulizumab
CD20	MS, Autoimmune encephalitis(AIE), Rheumatoid arthritis(RA)	Ocrelizumab
	MS, RA, Systemic lupus erythematosus(SLE), Anti-neutrophil cytoplasmic antibody-associated vasculitis	Ofatumumab
	SLE	Obinituzumab
	MS, NMOSD	Ublituximab
	Immune thrombocytopenia(ITP)	Veltuzumab
	RA, AIE, Graves' orbitopathy(GO), Myasthenia gravis(MG), Autoimmune blistering diseases(AIBDs)	Rituximab
CD22	SLE, Primary Sjögren's syndrome	Epratuzumab
CD25	MS	Daclizumab
CD28	RA, Juvenile idiopathic arthriti(JIA), SLE, Systemic sclerosis(SSc)	Abatacept
	RA	Belatacept
CD38	AIE, Immune thrombotic thrombocytopenic purpura (iTTP), Refractory autoimmune hemolytic anemia, SLE, Cold agglutinin disease, Autoimmune cytopenias	Daratumumab
CD49	SLE	Elotuzumab
	MS, RA, Crohn's disease(CD)	Natalizumab
CD52	MS, Sporadic inclusion body myositis	Alemtuzumab

DOI: 10.54254/2753-8818/21/20230855

Table 2. (continued).

IGF-1R	Thyroid eye disease, Thyroid orbitopathy, Thyroid-associated ophthalmopathy (TAO)	Teprotumumab (Tepezza)
α4β7integrin	CD, Inflammatory bowel disease(IBD), Ulcerate colitis(UC)	Vedolizumab
TNF-α	RA, JIA, Psoriatic arthritis(PsA), AS, CD, Ulcerative colitis, Psoriasis, Pyogenic sweat glands, Uveitis	Adalimumab
	CD	Infliximab
	RA, Polyarticular JIA, Systemic JIA	Tocilizumab
	CD, UC, IBD, SLE	Golimumab
	CD, UC, Axial spondyloarthritis (axSpA), IBD, Plaque psoriasis(PsO)	Certolizumab Pegol
IFN-α	SLE	Rontalizumab
	SLE, Idiopathic inflammatory myopathies (IIM)	Sifalimumab
	SLE	Rontalizumab Anifrolumab
IL-4/IL-13	Asthma, Atopic dermatitis(AD)	Dupilumab
	SSc	Romilkimab
IL-6/IL-6R	CD, RA, SSc, Autoimmune eye disease, AIE, GO	Tocilizumab
	NMOSD, Myelin oligodendrocyte glycoprotein (MOG)-IgG-associated disease (MOGAD)	Satralizumab
	CD, RA	Atlizumab Sarilumab
IL-17	AS, Oderate to severe psoriasis, Hypertrophic palmoplantar psoriasis, Generalized pustular psoriasis, PsA, RA, SLE	Secukinumab
	PsO	Bimekizumab
IL-23	PsO, Palmoplantar pustulosis(PPP), CD, PsA	Guselkumab
	PsO	Tildrakizumab Risankizumab
IL-12/23	PsO, PsA, Non-infectious uveitis (NIU)	Adalimumab
	UC	Mirikizumab
	PsO	Briakinumab
	CD, UC, IBD, Psoriasis, PsA, AD, SLE	Ustekinumab
FcRn	MG, AIE, Autoimmune bullous diseases, Bullous pemphigoid, Chronic inflammatory demyelinating polyradiculoneuropathy, Chronic Autoimmune Demyelinating Neuropathies, ITP, Autoimmune myositis, Pemphigus(Pemphigus Vulgaris ,Pemphigus Foliaceus)	Efgartigimab
Complement protein C5	AIE, ITP, MG	Rozanolixizumab
	AIE, MG, Catastrophic antiphospholipid syndrome(CAPS), Paroxysmal nocturnal hemoglobinuria(PNH), Atypical hemolytic uremic syndrome(aHUS), Thrombotic microangiopathy(TMA), NMOSD	Eculizumab
	Paroxysmal nocturnal hemoglobinuria(PNH)	Ravulizumab
vWF	iTTP	Caplacizumab
BAFF	SLE, SSc	Belimumab

DOI: 10.54254/2753-8818/21/20230855

6. Conclusion

Cancer and AIDs are both diseases caused by immune disorders, and in recent years, antibody therapies have become increasingly popular in the management of these two types of diseases, with mAbs being particularly outstanding. Despite the opposite pathogenesis of the two diseases, they share the same targets of action, which makes many mAbs have the potential to treat both diseases simultaneously. However, only a few mAbs have been studied for their "dual therapy" potential. At the same time, the side effects of mAbs are gradually being discovered, and the development of new mAbs for existing targets, the discovery of new specific targets, the design of bispecific antibodies, and the development of multi-drug combinations have become the direction of antibody therapies for cancer and AIDs. Future research is needed to further explore the potential of antibody therapies as "dual therapy" and develop new targets and drugs to bring more possibilities for clinical treatment.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230857

The application of biomimetic limbs in postoperative rehabilitation of amputees

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Abstract. Due to the inability to provide recovery opportunities, amputation is generally considered a failure of treatment. Traditional prostheses cannot interact with the surrounding environment, and the contact surface between the prosthesis and the residual limb is prone to wear and tear, causing pain to patients. Therefore, prostheses are generally regarded by patients as tools rather than a part of the body. In recent years, with the continuous development of artificial intelligence, materials science, and biomedical engineering technology, bionic limb technology has received widespread attention and application. Currently, bone integration and electromyography interface technology are widely applied in bionic limbs, allowing for more realistic and diverse options for prostheses for patients. Material innovation and machine learning technology can improve the mechanical performance, perceptual feedback effects, and autonomous learning ability of bionic limbs, but further research and exploration are still needed. This review paper aims to summarize and evaluate the current development status, application areas, and future research directions of bionic limb technology.

Keywords: bionic limbs, osseointegration, myoelectric interface, brain-computer interface.

1. Introduction

Amputation can have a significant impact on patients, causing not only secondary diseases in the residual limb but also psychological disorders due to changes in appearance and loss of basic abilities. Clinical experience shows that if a prosthesis fails to meet patients' expectations for appearance, function, and subjective considerations, it will eventually be rejected by the patient [1]. Although traditional prostheses can achieve multi-degree-of-freedom motion, users rely mainly on visual and pressure feedback from the socket at the interface between the residual limb and the prosthesis because of the lack of sensors, and they cannot alleviate phantom limb pain in some patients. The control of non-disabled limbs mainly relies on the transmission of limb movement and sensory feedback signals conveyed by neural fibers that dominate muscles, joints, tendons, and skin [2]. However, bionic limbs, through technologies such as myoelectric interfaces, brain-machine interfaces, and intelligent sensors, enable sensory feedback to be transmitted to the brain, making patients feel more realistic and natural.

2. Bionic limbs

Bionic prosthetic technology is an artificial limb technology that mimics the natural structure and function of limbs, utilizing advanced biomedical engineering, materials science, and artificial

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DOI: 10.54254/2753-8818/21/20230857

intelligence techniques. The aim of this technology is to help people with missing limbs regain movement and sensation and improve their quality of life and work.

Bionic prostheses typically include sensors and actuators to simulate biological sensing and motion capabilities. Sensors can receive external stimuli and convert them into electrical signals by measuring pressure or temperature to simulate touch or temperature sensation. Actuators can then convert these signals into mechanical motion, such as by using electric motors or hydraulic systems to simulate muscle contraction and relaxation.

Bionic prosthetic technology can achieve limb motion control and sensory feedback through various means, such as myoelectric interfaces, neural implants, visual feedback, and material innovation. Among these, myoelectric interface technology is the most widely used bionic limb control technology, which can convert limb motion into electrical signals through muscle electrical signal sensors and achieve precise limb control and motion sensory feedback. Neural implant technology can achieve more natural limb motion and sensory feedback, but requires highly complex surgery and post-operative management, limiting its promotion in clinical applications. 3D printing technology provides more diverse choices for patients to select prosthetic limbs [3]. The advancement of 3D printing technology can create models more rapidly, and lower the cost of developing prostheses by using more economical materials and low-cost printers, alleviating the difference in access to prosthetic care around the world [4].

The design and manufacture of bionic prostheses can be customized according to different needs and purposes, such as manufacturing arms, legs, hands, and feet, to help people with disabilities regain their ability to live independently. At the same time, the development of bionic prosthetics can promote the development and cross-application of fields such as biology, medicine, and robotics.

3. Osseointegration technique

3.1. Technical content

Osseointegration aims to firmly connect prosthetics with human bones. This technique involves implanting a small titanium alloy screw into the residual limb bone, and then directly attaching the external prosthesis to the titanium screw through subcutaneous tissue, achieving a strong bond between the bone and the prosthesis.

The primary benefit of using osseointegrated prosthetics is that they can provide more natural and stable mobility while reducing the wear and tear at the interface between the residual limb and the prosthesis. Compared to traditional prosthetics, patients using osseointegrated prosthetics can move and engage in activities more freely without the need for frequent adjustments or replacements.

Titanium alloy materials are mainly used in osseointegration [5]. Titanium alloy has high biocompatibility and can be used in the human body for a long time without rejection. However, surgery is not always successful, mainly due to aseptic loosening and infection of the implant [6], and osseointegration technology is not suitable for patients with osteoporosis, osteomyelitis, and other bone problems. Nevertheless, osseointegration technology is still in its relatively early stages and has vast potential for development with more application scenarios and technical solutions to be explored in the future.

3.2. Example

An Australian team conducted a rehabilitation and outcome-tracking protocol called OGAAP-1 on a total of 50 unilateral transfemoral amputees. The team offered two prosthetic options for bone-anchored reconstruction: Integrated Leg Prosthesis (ILP) and Osseointegrated Prosthesis (OPL) [7].

DOI: 10.54254/2753-8818/21/20230857

Table 1. Pre-and post-operative amputation mobility predictor [7].

Pre·and post-operative K-levels	Patients (n)
Improved	30
K0 to K2	2
K0 to K3	12
K0 to K4	1
K1 to K3	1
K2 to K3	11
K3 to K4	3
Unchanged	20
K2	2
K3	13
K4	5
Reduced	0

K0: The patient is incapable of securely ambulating or transferring with or without help, and a prosthesis does not improve their mobility or quality of life. K1: Patient is capable of using a prosthesis for transfers or ambulation on flat surfaces with a fixed cadence, such as a conventional restricted or unlimited household ambulator; A typical community ambulator, a K2-patient has the capacity or potential for ambulation and the ability to navigate low-level environmental barriers like curbs, stairs, or uneven terrain. K3: The patient has the capacity or potential for variable cadence ambulation, is a typical community ambulator capable of navigating most environmental barriers, and may engage in rehabilitative or exercise activities requiring the use of a prosthesis beyond simple locomotion; K4: Patient exhibits high impact, stress, or energy levels characteristic of the prosthetic demands of the youngster, active adult, or athlete, and has the capacity or potential for prosthetic ambulation that exceeds basic ambulation skills.

Of the 50 patients, 27 experienced complications after surgery, with 10 undergoing soft tissue revision surgery, 21 developing infections, and 4 experiencing falls (3 of whom had severe osteoporosis).

The chart shows that 60% of the patients had improved mobility, with most reaching K3 levels. According to the team's protocol, patients could achieve walking without assistive devices after approximately 4-5 months post-surgery. Patients reported significantly improved satisfaction, quality of life, and physical abilities. However, bone-anchored reconstruction is limited to patients with sufficient bone density and free of infection, and the technique still faces challenges such as sterile loosening and infection.

DOI: 10.54254/2753-8818/21/20230857

4. Electromyographic (EMG) interface technology

4.1. Technical content

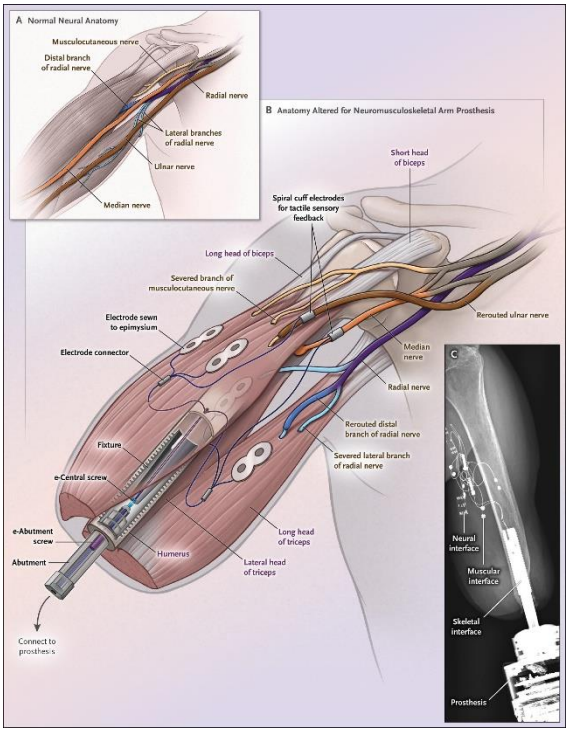

Figure 1. Neuromusculoskeletal arm prosthesis with targeted muscle reinnervation [10].

Electromyographic signals (EMGs) are the most widely used control signals in bionic limbs and have a history of 50 years [8]. Muscle-nerve interface (MNI) technology is a technique that detects muscle signals and transmits them to external devices. MNIs typically use electrode patches or arrays, while the motor and somatosensory cortices can interact with the residual limb. When generating a movement tendency, these electrodes can detect and capture contraction signals on the residual muscles of the patient, and convert them into signals that control external devices, thus achieving prosthesis control [9]. Amputation can affect the function and quality of residual limb muscle fibers, leading to atrophy, spasm, and/or pain. These problems can affect the quality of residual muscle activity and EMG signal in prosthesis control. Therefore, residual muscles must be stabilized with appropriate tension on muscles or bones [10]. Therefore, the type of surgical technique used in amputation is crucial for the preservation of muscle tension and length, recruitment of motor units, and proprioception.

4.2. Application example: self-sustaining neuro-musculo-skeletal prosthetic arm

A team led by Dr. Max Ortiz-Catalan performed a neuro-muscle-skeletal surgery on four patients in 2020, in which electrodes were sutured onto the fascia of the biceps brachia's two heads, as well as the long head and lateral head of the triceps brachii (Figure 1). These electrodes detect contraction signals of the residual limb to activate the prosthesis motor. Additionally, electrodes were placed near the ulnar and median nerves to deliver electrical stimulation into the nerve fibers to transmit tactile feedback signals on the prosthetic thumb [11].

DOI: 10.54254/2753-8818/21/20230857

5. Prosthetic sensing technology

5.1. Technical content

Prosthetic sensory feedback technology refers to the integration of sensors and feedback mechanisms into prostheses, enabling users to perceive and control the movement of their prostheses, thereby enhancing their function and adaptability. Sensory feedback is critical in daily life, as it eliminates the need for continuous visual monitoring, which is slower and more error-prone without feedback [12].

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5.2. Application example

A laboratory at MIT has developed more precise and stable prosthetic control through antagonistic muscle interface technology (AMI). Antagonistic muscles are a pair of muscles that work together around a joint; when one muscle contracts, the other relaxes [14]. AMIs connect the antagonistic muscle pairs around the residual limb, collect information on the length and force of the residual limb's stretching muscles during prosthetic use, and transmit this information to sensors. The sensors then send electrical signals to the central nervous system, allowing patients to perceive the prosthetic's force and impedance information and achieve a natural proprioceptive sensation [15].

6. Conclusion

This study analyzes the breakthroughs and experimental cases of bionic limb technology in various fields in recent years and argues that in the future, bionic limbs will make prosthetics more similar to natural limbs, both in terms of function and appearance. The clinical applications of osseointegration, cognitive biological screens, implanted sensors, advanced control algorithms, and myoelectric interface technologies will become more widespread. Osseointegration technology provides prosthetics with more degrees of freedom and has already been applied clinically. Compared to brain-machine interfaces (which control prosthetics through conscious thought), controlling prosthetics through myoelectric interfaces is easier to achieve. At the same time, interdisciplinary collaboration and communication need to be strengthened to promote the innovation and development of bionic limb technology to better meet humanity's growing health and lifestyle needs.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230858

An overview of various benefits of exercises other than weight control in obese adolescents

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Abstract. Obesity, with its increasing prevalence in adolescents, needs to be addressed with greater attention. As an effective measure to alleviate metabolic symptoms, physical activity and exercise have been widely studied in various studies, including different kinds of exercises. To study the benefits of exercise in various ways on obese adolescents, this literature review summarizes and discusses a few journals that test the effects of various exercises with the elimination of diet interventions. With the use of search engines like EBSCO, PubMed, Lancent, etc., several related research articles were selected with the key words "adolescent obesity", "physical activity", and "exercise". In conclusion, though there is still uncertainty about the minimum effective amount and specific types of exercise that are most beneficial, as well as the underlying mechanisms, research shows exercise is beneficial for overweight youth, though limitations in sample diversity may limit the validity of some studies. Future research should continue to explore effective exercise training for obese adolescents.

Keywords: adolescent, obesity, exercise, HIIT, physical activity benefits.

1. Introduction

Obesity, being associated with the increasing prevalence of various chronic health problems, manifests as a primary health issue. Over 1 billion individuals are fat worldwide, including 340 million youths. This result is shown in the 2022 report of the World Health Organization [1]. 19.7% of adolescents are affected by obesity in 2017 – 2020, with increasing prevalence among certain populations [2]. 41.9% of US citizens had obesity in 2017 – 2020 [3]. The medical cost of obesity was around $173 billion in 2019, and that for adults with obesity was $1861 more than that for healthily weighted people. Therefore, tackling obesity elicits both clinical and economic implications in the United States [4].

Counteracting such national pandemics, obesity, physical activity, any movement that expenses calories, is considered as an effective method. Sports training is a subcategory of sports activities. It refers to "planned, structured, and repetitive activities". Of course, it aims to improve physical fitness and health [5]." As obesity results from the overbalanced energy intake rather than energy expense, exercise training is widely used to ameliorate metabolic health in people with obesity: a most adjustable factor in behavioral modification that increases caloric output with increasing physical activity.

To treat obesity, effective weight loss continues to be the focus. However, the current obesity rate is still high, and ignoring the various benefits of physical activity leads to insufficient comprehensive control of energy input and output. Thus, it's crucial to investigate the benefits of physical activity

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DOI: 10.54254/2753-8818/21/20230858

independent of weight loss, for complementing physical and mental health for obese people. To study exercise would demonstrate what kinds of positive effect on obese adolescents and young adults' health, several academic journals were summarized and discussed.

2. Method

To discuss the various benefits of exercise in treating obesity, the search engine Google Scholar, PubMed, The Lancet, and EBSCO were primarily used to generate relative studies regarding the benefits of exercise for overweight youth (BMI > 25 kg/m^2 or greater than the 85th percentile, 10-20 years of age). The following terms were included when searching databases: obesity, exercise, physical activity, and adolescent obesity.

The criteria included articles that were peer reviewed academic journals, originally in English, published between 2017 and 2022, and that all participants were adults with average BMI > 30 kg/m^2. Since the obese BMI range is classified by the CDC, the selected studies excluded those participants are overweight (with the BMI < 30 kg/m^2) to maintain the consistency of the literature review. The research studies in the past five years are believed to show trends of study interests recently, with more up-to-date analysis technique. Studies that included dietary change or supplements intervention were excluded. For defining terms like physical activities and exercise, past journals before 2017 were taken into consideration. There were 89,234 academic journals in total on NYU Library EBSCO Discovery, and the studies were selected from the first three pages for most relativity. This literature review aims at compiling exercise's multidisciplinary effects on obese adolescents and young adults' comorbidity. Also, the commonalities of study research methods among these research were studied.

3. Results

Bagherniya et al., aiming at studying a school-based physical activity intervention's effectiveness while applying the theory of social cognitive, conducted a seven-month randomized controlled trial that included 172 girl students (from 12 to 16 years of age) categorized as either overweight or obese (BMI values are equal to or greater than 85thpercentile) who completed the study. In this trial, students from central Iran were separated into the intervention group (87 students) and the control group (85 students). The intervention group received school-based interventions. These interventions include sports workshops, regular private consultations, free practical and enjoyable exercise courses, free practical and competitive sports courses, etc. In contrast, the control group students received three physical education classes, while their parents and teachers were given one handout regarding the benefits of physical activities, in addition to 10 tickets to the nearest gym, which aims to provide control group benefits as well, in accordance with the social study ethical standards. Weight, height, and waist circumference were used as primary outcome measures, while psychological factors like self-efficacy, social support, outcome expectations, intention, and perceived barriers were used as secondary outcome measures. These measurements were taken using the valid and reliable SCT questionnaire. At the end, for the intervention group, the average BMI decreases 0.7 kg/m^2 from baseline 29.2 kg/m^2. The hours of sedentary behaviors changed from 3.2 to 2.8. Physical activity self-efficacy average value increased from 13.5 to 20.7. Physical activity intention mean value increased from 1.6 to 2.9. Perceived barriers decreased 6.5 from baseline of 25.1. Physical activity, social support, result expectations, and expectancies did not change significantly, though [4].

This experiment that S. Lee et al. conducted aims to compare the effectiveness of aerobic exercise, resistance training, or a combination of both. The study enrolled 118 overweight or obese adolescents with BMI greater than 85th percentile from 12-17 years old, who have a sedentary lifestyle. All of the participants recruited from the Children's Hospital of Pittsburgh were randomly divided into three groups for a period of 24 weeks: 38 were assigned to aerobic exercise (AE), 40 to resistance exercise (RE), and 40 to a combination of AE and RE. To guarantee the negative energy balance is solely from exercise intervention, nutrition counseling sessions were conducted for participants to choose proper food that meets the baseline calorie target. For the intervention, participants were suggested attending 60-minute sessions in a small group of 1-2 participants 3 times per week for 6 months. Total fat, fat-free

DOI: 10.54254/2753-8818/21/20230858

mass, body weight, waist circumference were measured using appropriate equipment respectively. After the study, 85 of 118 participants accomplished the program. The baseline information for each group did not significantly differ from one another. For the primary outcome, though insulin-stimulated glucose disposal of all groups increased hugely, not different from the combined group, Rd improvement in AE group was greater than in the RE group. All groups had a great reduction in 2-hour glucose test, notwithstanding the fasting glucose level for all groups did not alter. For secondary outcomes, only the AE group showed significant reductions in BMI (-1.4 ± 0.1 kg/m^2) and waist circumference (-3.2 ± 0.2 cm). The weight change in the AE group was greater than that in the combination group [6].

Following their first randomized controlled trial, Lee et.al conducted a secondary study to further explore exercise modality on regional body fat and CVD markers. Posters in Pittsburgh were used to enlist 118 adolescents (12–17 years old) who were overweight (the body mass index (BMI)≥85th percentile and <40 kg/m^2; N = 118). Three times a week for 60 minutes, participants in the aerobic group were required to participate in exercise training sessions. Aerobic exercise, progressive in duration and intensity, includes treadmills and/or ellipticals. There is a 1-2 minute break between the two groups. Resistance training includes two groups. Each group repeats 12-15 times. A total of 8 full body resistance exercises. In the combined group, participants were asked to do a 30-minute treadmill and/or elliptical, and 1 set of resistance exercise. At the end of the study, 118 eligible individuals participated in the intervention, while 30 of 38, 28 of 40, and 27 of 40 participants from aerobic, resistance, and combined groups respectively completed the study. Exercise training and cardiovascular disease risk markers did not differ significantly from one another. Only the aerobic group showed a statistically significant reduction in both local and total subcutaneous adipose tissue (SAT). The results revealed that the changes in the aerobic group were substantially bigger than those in the combination group for the lower body SAT ($p=0.02$). In contrast, significant body weight reductions were observed in all groups ($p < 0.05$) [7].

In order to determine the effects of 12 weeks of high intensity interval training (HIIT) and equal intensity continuous training (MICT) on the decrease of abdominal visceral fat in overweight young women (18-22 years old, BMI 25) (kg/m^2), Zhang H et al. conducted the experiment. The percentage of body fat measured by double X-ray absorption method (DEXA) is greater than 30%. In the study, 52 female university students signed up and 47 enrolled. 15 students served as controls with no training, and 16 each were randomly assigned into HIIT and MICT groups, each receiving identical amounts of training. Every participant's diet was recorded from 3 weeks before the training began and during the 12-week training period. A CT scanner was used to measure the visceral and subcutaneous fat areas of the abdomen. 43 of 47 participants finished the training program, for which 15 of 16 individuals from MICT and HIIT groups, and 13 of 15 from the control group. The reduction in body mass (HIIT Group Before 67.3 ± 6.1, after 64.0 ± 6.0; MICT Group Before 68.5 ± 8.0, after 65.1 ± 7.7 $p < 0.01$) and body fat percentage (HIIT before 38.1 ± 2.3 after 35.6 ± 2.0; MICT before 38.0 ± 2.1, after 35.6 ± 2.3, $p < 0.01$) was observed (Figure 1). Decrease in total and regional body fat was also observed ($p < 0.05$). Overall and local body fat also decreased. This reduction is reflected in the number ($p<0.05$). AVFA showed a decrease in abdominal visceral fat in both groups ($p<0.05$), although compared to the control group. Also, participants in both groups showed an increased aerobic fitness after the 12-week training. Both types of exercise showed effective reduction in body fat mass, regional body fat mass, and abdominal visceral fat. However, the results showed no significant difference between MICT and HIIT in reducing body fat. Such results revealed the efficacy of exercise in reducing whole and regional body fat [8].

DOI: 10.54254/2753-8818/21/20230858

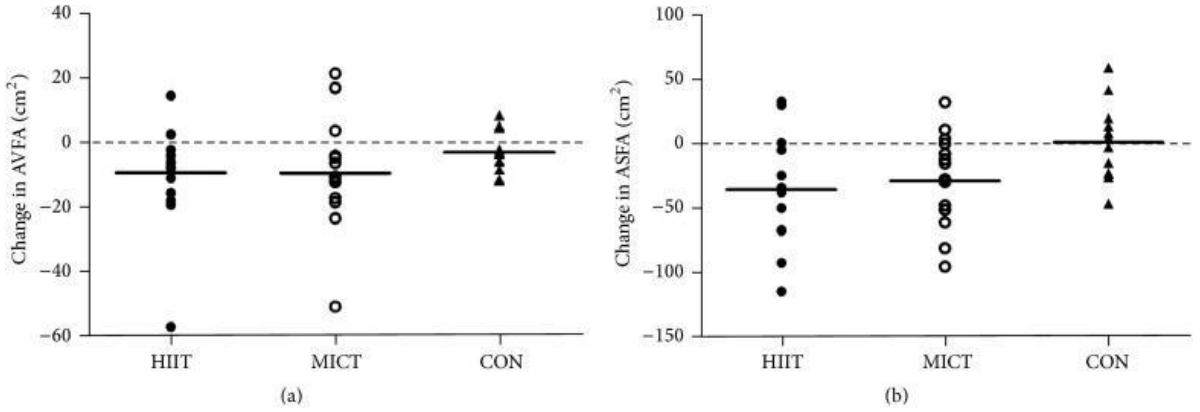

Figure 1. Changes in (a) abdominal visceral fat area (AVFA) and (b) abdominal subcutaneous fat area (ASFA) of participants post intervention in HIIT, MICT, and CON groups [7].

A randomized controlled experiment was carried out by Zhang L et al. The goal of this study is to determine how high-intensity intermittent exercise (HIIE) and high-intensity continuous exercise (HICE) affect inhibitory function in overweight children. The obese children selected here are 10-14 years old, with a BMI equal to or greater than 25kg/m^2. The level of inhibitory function is measured right before and after the intervention by the Stroop test, a.k.a Color Naming Task, often used to assess not only cognitive function but also inhibit habitual responses. The HIIE group was asked to do a 30-min treadmill session. The HICE group was asked to complete 30-min 80-85% maximal heart rate rope skipping exercise. The control group was asked to watch a designated cartoon for 30 minutes. The results found were compared. The results showed significant differences between the HIIE group (1.69 ± 0.21 seconds) and the CON group (0.43 ± 0.21 seconds) and between the HICE group (1.85 ± 0.21 seconds) and the CON group. In contrast, there was no significant difference between the HIIE and HICE groups ($p = 1.00$). Strengths include the relatively large sample size with both genders and pathbreaking to test relationship between inhibition function and exercise. Limitations include the single method to test executive cognition and inhibitory function, and lack of documenting heart rate during the intervention. Future studies could research the mechanism between acute HIIE and cognitive functions [9].

As previous studies showed the time-efficient property of circuit resistance training (CRT), and higher metabolic rate than traditional resistance training (or a combined one with aerobic training), Rasooli et al. recruited 40 adolescent boys aged 14-17 years with BMI >= 95%, to study the effect of CRT on levels of improvement in insulin resistance (IR) related metabolites. Control and CRT-intervention groups, each with 20 participants, were randomly assigned to the participants. The intervention group received CRT for 8 weeks, and were asked to avoid exercise outside the study and maintain their usual diet to prevent confounding effects. To be more specific, the CRT program includes briefing sessions, introducing the 1-repetition maximum protocol and various exercise devices. The protocol involves low-moderate intensity activities and warm-up repetitions. After that, participants were asked to perform 10-12 rounds of lifting with an elevated intensity. The results were collected via plasma and urine sample, as well as statistical analysis. In the end, the training group (n=16) showed decreases in post-intervention data of serum profile: glucose, insulin, total testosterone, etc. Also, anthropometric characteristics showed decrease in general, except fat-free mass entailed an increase [10].

4. Discussion

Overall, the studies chosen for this literature review have varied in intervention time from a one-time test up to 30 minutes to a long-term intervention of 10 to 18 weeks. All studies exclusively studied the effect of various exercise training session with control in participants' regular diet. Though varied in actual effects, after intervention, participants from the experimental group manifested positive effects in

DOI: 10.54254/2753-8818/21/20230858

reducing anthropometric data, metabolites associated with the metabolic syndrome from blood or urine tests, and improving psychological factors like self-esteem and inhibitory functions.

Notwithstanding, advantages and disadvantages entailed by the study designs should also be recognized for more comprehensive study designs in the future. For example, strengths of the study by M. Bagherniya et al. includes special activity programs for students, parents, and teachers at school base. Limitations include: 1. Subjectiveness of self-reported questionnaire; 2. Un-matched randomization between two groups due to the random sampling method used only for selecting eligible schools. Strengths include using gold-standard MRI to measure body composition and fat distribution. Limitations include that the results cannot be generalized to public adolescents due to varied accessibility to exercise trainers and fitness centers. Also, results in other ethnic groups are unknown. Moreover, strengths of the study of S. Lee et al. enclose randomized study design, great adherence to exercise regimen (90%), direct supervision and monitoring. Limitations include the relative lack of certain ethnic group participants like Asian youth, no non-exercising control group, 64% of participants were female, and possibility of non-generalizable results.

5. Conclusion

Presently, research shows benefits of exercise, both aerobic and resistance training, in a myriad of fields, from mental development to body composition and alleviation of comorbidity, including body composition, metabolic syndrome related metabolites level, insulin resistance, and so forth. However, specific limits like the minimal effective amount of exercise and specific types of exercise that are more efficient have yet to be defined, neither the mechanisms between exercise nor benefited organisms. In short, exercise is beneficial to health of overweight youth. For now, some of the research has limited inclusion of sample variety in gender, ethnic group, region, etc., which may hinder their validity for a larger population. Future researchers may continue the path in exploring more effective exercise training that could positively affect obese adolescents. Admittedly, this literature review has its limitations in comprehensiveness with a few number of articles reviewed. However, it would be helpful in setting future research areas by summarizing existing research and their methods.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230859

Application of psilocybin in mental health disorders

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Abstract. Psilocybin is a naturally occurring psychoactive compound, which has been used for ages in traditional settings for religious and therapeutic use. Recent studies have renewed interest in psilocybin for its potential therapeutic benefits in treating depression and anxiety. The pharmacodynamics of psilocybin are complex, involving its rapid conversion to psilocin and its activity on various serotonin receptors, particularly the 5HT2A/C and 5HT1A receptors. In addition, psilocybin can increase glutamate release, which is believed to be an important mechanism underlying its therapeutic effects. Clinical trials have demonstrated that psilocybin has a long-lasting antidepressant effect, with little to no side effects. However, it is necessary to further study the mechanisms underlying its therapeutic potential and to optimize its use in clinical settings. Overall, the promising findings suggest that psilocybin may offer a valuable alternative to traditional antidepressant therapies for individuals suffering from depression and anxiety. Meanwhile, studies have shown that this drug also has certain benefits for mental disorders such as addiction and obsessive-compulsive disorder. Thus, it is necessary to continue exploring the potential of psilocybin as a novel strategy in treating mental health disorders.

Keywords: psilocybin, mental health disorders, application.

1. Introduction

Psilocybin, a prominent hallucinogenic substance, is mainly obtained from certain species of mushrooms found globally. However, the misuse of these drugs has led to detrimental effects among a growing number of psychedelic drug users, highlighting the need for careful consideration of its usage and effects [1].

The primary active metabolites of psilocybin, psilocin, and psilocybin is a predominant agonist on serotonin 5HT2A/C and 5HT1A receptors, while the 5HT2A receptor agonism is believed to be essential for hallucinogenic effects. This mechanism of action underpins the drug's psychoactive effects, which have been studied in the context of brain function and experimental therapies in the field of psychiatry since the mid-twentieth century [2]. However, the use of psilocybin in these studies fell out of favor in the 1960s, only to be revived in the early 21st century.

More and more studies have shown that as a hallucinogenic agent, psilocybin extract has relatively low addiction and fewer adverse reactions. Its combination with psychological support has a significant effect in treating depression, anxiety, substance addiction, obsessive-compulsive disorder, and other aspects. Among them, psilocybin extract has been granted breakthrough therapy status by the US FDA in treating refractory depression and severe depression. Psilocybin can trigger changes in

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DOI: 10.54254/2753-8818/21/20230859

the brain that result in an overall decrease in brain activity, which can lead to a decrease in symptoms of depression. While the effectiveness of psilocybin on depression has already been demonstrated through experiments, its effect duration and side effects compared to traditional depression drugs require further investigation.

This review aims to explore the mechanisms of psilocybin, with a focus on its therapeutic effects on depression in clinic. By exploring existing the research data and theoretical perspectives, this review will provide insights into the potential use of psilocybin as a promising therapy for depression, while also highlighting areas for future study and exploration, and prospect the current research hotspots, in order to provide reference and reference for relevant basic research and clinical treatment.

2. Chemical structure and metabolism

Psilocybin is a natural compound that exists in over 200 species of fungi, of which the psilocybe genus is the most potent source. Upon ingestion, Psilocybin undergoes rapid dephosphorylation in the intestinal mucosa to produce psilocin, which is considered the primary active metabolite of psilocybin. This process is facilitated by alkaline phosphatase and nonspecific esterase enzymes. Studies involving rats have shown that approximately 50% of the total volume of psilocin is absorbed from the digestive tract after ingestion. Then, through endoplasmic enzymes, specifically UDP-glucuronosyltransferase (UGTs), Psilocin is further metabolized to psilocin-O-glucuronide, which accounts for about 80% of the excreted form of psilocin.

The onset time of psilocybin extract is very fast, and after 15 minutes of oral administration, it can produce some subjective effects, such as feelings of happiness, happiness, and satisfaction. It reaches its highest blood concentration within 100 minutes, with a half-life of about 160 minutes, and its efficacy shows a significant dose-dependent effect. However, there is no clear consensus on the optimal therapeutic dose of naked mushroom extract in current research, The commonly used dosage in foreign clinical trials is based on a body weight of 0.3-0.429 mg/kg. In an animal experiment, it was found that psilocybin reached its maximum plasma level approximately 90 minutes after administration, and then distributed to all tissues, including the brain, which is also in line with common clinical situations. Interestingly, prior to accumulating in the brain, psilocin accumulates in the liver and kidneys. The precise mechanisms involved in the uptake, distribution, and metabolism of psilocin remain to be fully elucidated, which is an ongoing research field [3,4]. Nonetheless, understanding the pharmacokinetics of psilocybin and its active metabolite, psilocin, is crucial for the safe and effective use of psilocybin in therapeutic settings.

3. Pharmacodynamics

Psilocybin and psilocin are two indoleamine compounds that have been widely investigated for their pharmacological properties, including their predominant agonist activity on serotonin receptors. Specifically, psilocybin and psilocin are known to exhibit agonist activity on the 5HT2A/C and 5HT1A receptors, with 5HT2A receptor agonism being vital for the development of hallucinogenic effects [2].

Psilocin has been found to have a broader receptor-binding profile than psilocybin, with weak affinity for receptors such as Imidazoline1, Alpha2A/B/C, and 5HT transporters, as well as dopamine receptors [5]. Research has suggested that psilocin may increase glutamate release through presynaptic 5-HT2ARs located on thalamocortical terminals in the neocortex, thereby contributing to its hallucinogenic properties.

Recent studies have also revealed that psilocybin can modulate dopamine release in humans, likely via 5HT receptor-mediated mechanisms. Specifically, psilocybin has been reported to indirectly improve the release of dopamine in the ventral striatum, which was found to be correlated with symptoms of depersonalization and euphoria [6].

Additionally, psilocybin has been shown to inhibit dorsal raphe nucleus activity via 5-HT1A autoreceptors[4]. This modulation of 5-HT1A autoreceptor activity is thought to contribute to psilocybin's antidepressant effects, which have been observed in several clinical studies.

DOI: 10.54254/2753-8818/21/20230859

Also, psilocybin has been shown to affect gene expression in the brain. Specifically, it has been shown that psilocybin can increase the expression levels of early genes, which can be rapidly activated by a variety of cellular stimuli, including erg-1, erg-2, c-fos, jun-B, period-1, gpcr-26, fra-1, N-10, and I-κBα. Psilocybin has also been found to reduce the expression of sty-kinase, a protein involved in cellular signaling pathways [7]. However, the exact signaling pathway leading from receptor activation to the modulation of early gene expression remains to be fully understood, thus requiring further investigation.

In conclusion, the complex pharmacological actions of psilocybin and psilocin on various receptor systems, neurotransmitter pathways, and gene expression suggest their potential as therapeutic agents for a range of neuropsychiatric disorders. Further studies are needed to fully elucidate their mechanisms of action and clinical efficacy.

4. Effect duration

4.1. Depression

As a natural compound existing in certain species of mushrooms, psilocybin has been noted for its potent hallucinogenic effects. However, recent research has demonstrated that psilocybin may also have therapeutic benefits in treating depression. In a groundbreaking open-label study, a cohort of 12 participants (six males and six females) with moderate-to-severe depression were administered two doses of psilocybin (10 mg and 75 mg) and were monitored for six weeks [8]. The study employed a brief inventory of depressive symptoms to assess levels of anxiety in participants before and after treatment. The study's results were highly encouraging. After receiving psilocybin treatment, participants reported a significant reduction in the activeness of negative emotions, suggesting psilocybin's potential efficacy as a tool for managing depression. Intriguingly, all participants reported similar experiences of an emotional "confrontation" during treatment sessions. Such emotional breakthrough and resolution are believed to stem from the recovery of past traumas, which participants could confront and work through in their psilocybin sessions.

The study represents a notable advancement in the understanding of psilocybin's potential therapeutic benefits. While further studies are needed to establish its mechanisms of action and long-term effects, this study provides compelling evidence supporting psilocybin's potential value in treating depression. It opens up new avenues for research into the area, presenting a promising opportunity to explore the intersection of psychedelics and mental health treatment.

There was an observable effect on depression severity in the first week after taking psilocybin, indicating its effectiveness. The effect of the drug reached its peak in about two weeks, and then decreased, but it was still effective after three months [9].

The long persisting therapeutic effects after acute drug effects challenges biological theories of classic therapeutic psychedelic effects, and a possible hypothesis is that psilocybin acute destabilizes brain networks, and it may have the occasion to alter brain network activity persistently [10]. Clinical trial also shows that the result can remain significant 6 months post-treatment in a treatment-resistant cohort [11].

Recently, psilocybin has gained growing attention as a potential therapeutic agent for various mental illnesses, including depression, anxiety, and addiction. Emerging evidence suggests that psilocybin has a longer-lasting therapeutic effect compared to conventional medications used for treating these conditions. For instance, a study investigated the long-term effects of psilocybin therapy on depression and anxiety, and found that a significant therapeutic effect was observed up to 12 months after treatment [11]. The study involved 27 participants who were randomized into immediate and delayed treatment groups, and the results showed that all participants had higher baseline GRID-HAMD scores at the 12-month timepoint, except for three subjects who did not meet the treatment response criteria at any timepoint after treatment.

Moreover, a study reported that within 4.5 years after patients with cancer-related distress received treatment, psilocybin treatment showed a significant decrease in depressive symptoms, indicating a

DOI: 10.54254/2753-8818/21/20230859

possibility of a longer-lasting effect of psilocybin treatment in major depressive disease [12]. A phase II clinical trial involving 233 patients with the severe depression who received different doses (1mg, 10mg, 25mg) of psilocybin for 3 weeks. The results showed that a single dose of 25mg (not 10mg) psilocybin showed the significant decrease in the depression scores compared to 1mg after 3 weeks of treatment, but may also be accompanied by larger side effects [13].

In a clinical trial, 19 patients with intractable depression were included, and they were treated with 25 mg psilocybin in combination with psychotherapy. At the same time, the emotional face task was used to test the response of the patients to different facial stimuli. After treatment, the depression of the patients was significantly reduced, and the response of the right amygdala nucleus to fear and neutral facial stimuli was significantly increased, and the change of the amygdala nucleus stimulus response was significantly related to the improvement of depression symptoms. It shows that the mechanism of action of psilocybin on amygdala is different from that of traditional antidepressants, due to the mechanisms of action of traditional antidepressants is to reduce the response of amygdala nucleus to negative stimulation [14]. More clinical trials with different doses and times are needed to further verify its efficacy and safety.

4.2. Addiction

In addition, psilocybin has also been found to have a long-term effect in suppressing addiction, particularly smoking cessation. A study involved 15 smokers, including heavy smokers who smoked an average of 19 cigarettes per day over the past 31 years [15]. The subjects received a 15-week combination treatment including cognitive-behavioral therapy (CBT), elements of mindfulness training, and guided imagery for smoking cessation. The subjects received a moderate dose of psilocybin at week 5 of treatment, which served as the Target-Quit Date (TQD), and a high dose of psilocybin approximately 2 weeks later. After the study, at the 12-month follow-up, 10 participants (67%) were biologically confirmed to be quitters. This finding is further supported by previous studies suggesting high rates of positive results of psychedelic-facilitated treatment of alcoholism. Therefore, the effectiveness of psilocybin in treating addiction can be considered significant, and research is required to further explore its potential in this area.

A systematic review of four clinical trials combining psilocybin with psychotherapy found a significant positive effect of therapy which psilocybin is used in subjects with either addiction of tobacco or alcohol use. Three of the trials were single-arm pilot studies with a substantial risk of bias, but one trial was a double-blind, placebo-controlled randomized controlled trial (RCT).

The proposed mechanisms by which psychedelics, including psilocybin, improve the symptom of addiction are both biological and psychological. Biological mechanisms include inducing brain neuroplasticity via ascending the levels of brain-derived neurotrophic factor (BDNF), which are reduced under psychiatric conditions. Psychological mechanisms include inducing a experience of mystical during the psilocybin session, which seems to cause behavioral change in patients with addiction [16].

4.3. Obsession

Nine adults (seven males and two females) with prior experience with psychedelic drugs and symptomatic OCD were provided with repeated psilocybin doses in a clinical trial. On average, the participants did not show improvement even after receiving 3.4 rounds of medication that are typically used to treat obsessive behavior. The Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) is employed to investigate the severity of symptoms, and all participants signed informed consent. Psilocybin reported to cause a psychedelic experience in a dose-dependent manner, and no participants experienced psychotic symptoms or dangerous behaviors. The most significant observation during the clinical study was the sudden decrease in OCD symptoms in all participants during one or more testing sessions. The extent of improvement varied from minor to complete, but it was temporary [17].

In summary, as a potential drug for treating depression, anxiety, and addiction, psilocybin has shown promising results. Its long-lasting therapeutic effects have been observed up to 12 months after

DOI: 10.54254/2753-8818/21/20230859

treatment for depression and anxiety, and even longer in some cases. These findings provide a solid foundation for future research on the potential of psilocybin in treating mental illnesses and addiction, while caution should still be taken for its clinical applications, given its controlled substance status.

5. Side effect

Among psychedelic drugs, psilocybin is considered to be the safest. A wealth of past evidence and modern scientific research have shown that psilocybin is not highly toxic, not addictive, and does not have any sustained harmful physiological or psychological effects during or after use. It is also known to have safe psychological responses [18].

The low physical harm has been proven by complicated comparison [19,20]. However, the side effects were still found in clinical trials.

Psilocybin has an acute psychedelic effect. The effects of the drug usually became noticeable within 30 to 60 minutes after taking it, reached their highest point between 2 and 3 hours after ingestion, and eventually decreased to a point where the patient could be discharged and evaluated safely at least 6 hours after taking the drug [20]. According to a survey study that examined the impact of psilocybin consumption, 10.7% of participants reported behaviors that put themselves or others in danger of physical harm. Additionally, 2.6% of participants reported experiencing physically aggressive or violent behavior, while 2.7% of participants sought medical treatment from a hospital or emergency department during the research period [21]. While some of the participants with suicidal ideation reported reduced related thoughts, the other ones that have pre-existing suicidal thoughts or depression experienced more severe imagination on suicide. Two of them attempted suicide but was unsuccessful, and in total, a third of them reported suicide attempt besides two reporting severe suicidal ideation during the session. Three of the participants who were psychiatrically healthy before the psilocybin experience went through psychotic symptoms, including hallucination, depersonalization, very disturbing visual hallucination, etc. Each of them was subsequently diagnosed with psychotic conditions such as schizophrenia, bipolar disorder, and post-traumatic stress disorder (PTSD).

Another study showed that typical adverse events after taking psylosibin were transient anxiety (mostly mild) during drug onset, temporary thought disturbance or confusion, mild and temporary nausea, and temporary headache [11]. In addition to that, visions of an autobiographical nature were reported by 14 out of 20 participants, but the patient subsequently improved after open and compassionate listening was maintained. This evidence suggests that, despite the therapeutic effect on psychotically healthy people as mentioned previously, the ingestion of psilocybin could result in the exacerbation of mental illness or even the onset of mental illness. However, these appeared side effects are considered mild, and limited to a short period after dosing [22].

6. Conclusion

Psilocybin, one of the naturally occurring psychedelics found in certain species of mushrooms, has shown promising therapeutic effects in treating anxiety and depression, making it a popular focus of research in the field of mental illness treatment. The current body of research suggests that psilocybin may have considerable therapeutic potential, as it produces effects that last up to three months and has shown superior efficacy in managing moderate to severe depression compared to other available drugs, while also causing fewer and milder side effects.

The recent emergence of psilocybin as a possible treatment for depression is particularly exciting, given the limitations of currently available therapies. Traditional antidepressants, for instance, can take weeks or even months to produce a noticeable effect and can be accompanied by a series of adverse effects, including weight gain, sexual dysfunction, and gastrointestinal disturbances. On the contrary, psilocybin has been confirmed to produce rapid and lasting therapeutic effects, making it a potentially powerful tool for treating depression.

Despite these promising findings, psilocybin remains a Schedule I controlled substance under the 1971 United Nations Convention on Psychotropic Substances. This legal classification poses a

DOI: 10.54254/2753-8818/21/20230859

significant obstacle to conducting further research and clinical trials on the application of psilocybin, as it restricts access to the drug and limits its availability for research purposes.

While psilocybin has shown great potential, it is not without risks. Negative side effects have been observed in some patients, including temporary increases in anxiety, paranoia, and dissociation. It is also important to note that psilocybin can have powerful and potentially unpredictable effects on the mind, and as such, it should be used with caution in clinical settings.

Nonetheless, the promising treatment effects of psilocybin in treating the mental disorders have resulted in a reignited interest in the drug, and more research is being conducted to better understand its mechanisms of action and potential clinical applications. With further research, psilocybin may be regarded as a useful tool in treating mental illness, providing new hope for patients suffering from depression and other conditions.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230860

Protein folding and mental health: The impact of protein misfolding on neurological disorders

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Abstract. Major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SCZ) are psychiatric disorders that significantly impact an individual's ability to function effectively within society. MDD is characterized by persistent low mood, leading to symptoms such as anhedonia, feelings of worthlessness, and cognitive impairments. BD, another mood disorder, is typified by recurrent episodes of depression and mania or hypomania. The disorder is associated with increased risk of suicide, comorbid psychiatric conditions, and impaired functioning across various domains. SCZ, a severe psychiatric disorder primarily characterized by hallucinations, delusions, and disorganized thinking, affects approximately 1% of the global population. The cognitive, social, and occupational impairments associated with SCZ impose a substantial burden on affected individuals and their families. Even though it sounds like three completely different mental disorders, because of the high degree of matching of symptoms, we firmly believe that there is a relationship between these mental disorders. Protein folding is crucial for proper protein function, and misfolding can lead to neurodegenerative disorders. Through recent research findings, we found that the changes in Aβ plaques and tau tangles appeared in the typical psychiatric diseases mentioned above. This paper explored the impact of protein misfolding, changes in Aβ plaques, and tau tangles on neurological disorders, with a focus on depression, bipolar disorder, and schizophrenia, also examines the role of molecular chaperones in preventing misfolding and the impact of protein misfolding.

Keywords: major depressive disorder, bipolar disorder, schizophrenia, protein folding, Aβ plaques and tau tangles.

1. Introduction

Protein folding is critical for proper protein function, and misfolding can lead to neurodegenerative disorders. Recent research suggests a link between protein misfolding and mental health disorders, such as MDD, BP, and SCZ [1].

Over 264 million people worldwide suffer from major depressive disorder (MDD), one of the most widespread mental health conditions. A persistently depressed mood, loss of interest in or enjoyment from activities, feelings of hopelessness, and physical symptoms including weariness and sleep difficulties are some of its hallmarks.

Extreme mood swings with episodes of mania and sadness are hallmarks of bipolar disorder (BP), often referred to as manic-depressive illness (MDI) [2].

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DOI: 10.54254/2753-8818/21/20230860

Schizophrenia (SCZ) is a severe mental health disorder affecting approximately 20 million people globally which characterized by symptoms such as hallucinations, delusions, disorganized thinking, and negative symptoms like reduced emotional expression and motivation. Schizophrenia typically emerges during late adolescence or early adulthood and can significantly impact an individual's ability to function in society, maintain relationships, and work.

This paper will explore the impact of protein misfolding on neurological disorders, with a focus on the specific details of depression, bipolar disorder, and schizophrenia, and the role of molecular chaperones in preventing misfolding.

2. Protein folding and its importance in biology

Protein folding is a fundamental process in biology that is crucial for the proper functioning of living organisms. Proteins are responsible for a wide range of biological functions, such as catalyzing chemical reactions, transmitting signals, and providing structural support. The ability of proteins to carry out these functions is dependent on their three-dimensional structure, which is determined by the process of protein folding.

The complicated and dynamic process of protein folding, also referred to as the folding pathway, takes place in a number of intermediate states. A linear polypeptide chain is first synthesized, and it is subsequently folded to form a particular three-dimensional shape. The stability and shape of the finished protein structure are determined by a variety of molecular forces, including hydrogen bonding, electrostatic interactions, and van der Waals interactions throughout the folding process [3].

According to the Anfinsen's thermodynamic hypothesis, a protein's natural structure is determined by its amino acid sequence and the state with the lowest free energy. In other words, a protein's amino acid sequence encodes the knowledge required for the protein to fold into the correct three-dimensional shape. Protein function depends on appropriate folding, and improperly folded proteins can become toxic or lose their intended function, which can result in a variety of illnesses [4]. Alzheimer's, Parkinson's, and prion disorders are a few examples [5].

Protein folding occurs in a complex cellular environment, where many factors can influence the folding process. Molecular chaperones, for example, are proteins that assist in the folding of other proteins by preventing misfolding and aiding in the correct folding process [6]. Post-translational modifications, such as phosphorylation and glycosylation, can also play a role in protein folding by altering the structure and stability of the protein.

Despite the sophisticated mechanisms in place to ensure proper protein folding, misfolding can still occur. Misfolded proteins can be non-functional or even toxic to cells, leading to a range of diseases. For example, amyloid-beta protein misfolding is a hallmark of Alzheimer's disease, while alpha-synuclein misfolding is associated with Parkinson's disease [7]. In addition to neurodegenerative diseases, protein misfolding has also been implicated in cancer, cystic fibrosis, and other diseases [8].

Protein misfolding is recognized by quality control mechanisms in the cell, which can either repair or degrade the misfolded protein. For example, the chaperone-mediated autophagy pathway is a quality control mechanism that selectively targets misfolded proteins for degradation [9]. Other cellular mechanisms, such as the unfolded protein response (UPR), can also be activated to mitigate the damage caused by misfolded proteins.

3. Protein misfolding and mental disorder

Recent studies have suggested that protein misfolding may contribute to the development of mental health disorders [1]. Misfolded proteins can disrupt the normal functioning of neurons and alter neurotransmitter release, leading to cognitive and emotional impairments [10]. This section will explore the specific examples of MDD, BP, and SCZ in relation to protein misfolding.

3.1. Depression

Studies have suggested that misfolded proteins can contribute to the development of depression through the dysregulation of synaptic transmission, neuroplasticity, and neurogenesis. One study found

DOI: 10.54254/2753-8818/21/20230860

that the misfolding of brain-derived neurotrophic factor (BDNF) impairs its ability to promote neuronal growth and survival [11]. Furthermore, protein aggregates have been observed in the brains of patients with major depressive disorder, including increased levels of aggregated alpha-synuclein and ubiquitin-positive inclusions [1].

Protein misfolding may play a role in the deregulation of the immune system, which has been linked to the emergence of depression. Inflammation and immune system activation brought on by misfolded proteins have been connected to the etiology of depression, according to studies. The endoplasmic reticulum (ER), a cellular organelle involved in protein synthesis and folding, can also accumulate misfolded proteins as a result of protein misfolding. Unfolded protein response (UPR), which can cause neuronal malfunction and cell death in the brain and contribute to the onset of depression, has been found to be activated by ER stress brought on by misfolded proteins.

3.2. Bipolar disorder

Protein misfolding has been implicated in the pathophysiology of bipolar disorder. The disorder is characterized by episodes of mania and depression, and it is believed to arise from a combination of genetic and environmental factors. Evidence has shown that protein misfolding can play a role in bipolar disorder by altering the expression of proteins involved in synaptic plasticity and neurotransmitter release, such as DISC1 and GSK-3β [1]. Post-mortem studies of patients with bipolar disorder have also shown an increased presence of protein aggregates in the brain, including amyloid-beta plaques and tau tangles [10]. These findings suggest that misfolded proteins may contribute to the development of the disorder.

In addition to the altered expression of proteins involved in synaptic plasticity and neurotransmitter release, studies have shown that protein misfolding may also play a role in mitochondrial dysfunction, which has been implicated in the pathogenesis of bipolar disorder [12]. Mitochondria play a critical role in energy production and calcium signaling within neurons. Dysfunctional mitochondria can lead to an imbalance in calcium signaling and oxidative stress, which can contribute to neuronal damage and death. Research has shown that protein misfolding can lead to the accumulation of misfolded proteins in mitochondria, impairing mitochondrial function and contributing to the development of bipolar disorder [13].

3.3. Schizophrenia

Protein misfolding has been implicated in schizophrenia, with evidence showing altered expression of proteins involved in synaptic transmission and neuroplasticity, such as neuregulin 1 (NRG1) and dysbindin (DTNBP1) [1]. Post-mortem studies have demonstrated an increased presence of protein aggregates in the brains of schizophrenia patients, including amyloid-beta plaques and hyperphosphorylated tau, suggesting a potential role of misfolded proteins in the pathophysiology of the disorder [12]. Protein misfolding may contribute to the dysfunction of glutamatergic signaling in the brain, which has been implicated in the pathophysiology of schizophrenia. Glutamate is a neurotransmitter that plays a key role in synaptic plasticity and cognitive function. Research has shown that misfolded proteins can interfere with the proper folding and trafficking of glutamate receptors, leading to altered glutamate signaling and synaptic dysfunction in schizophrenia. In addition, protein misfolding can also lead to the accumulation of misfolded proteins in the extracellular space, which can activate the immune system and induce an inflammatory response in the brain. This neuroinflammation has been linked to the pathogenesis of schizophrenia and may contribute to the development of the disorder.

Recent advances in proteomics have enabled researchers to identify changes in protein expression and folding patterns in patients with neuropsychiatric disorders. By understanding the mechanisms underlying protein misfolding and aggregation, researchers hope to identify new targets for the development of more effective therapies for these conditions. In summary, protein misfolding is a critical factor in the pathogenesis of neuropsychiatric disorders, and its study may lead to the development of novel treatments for these debilitating.

DOI: 10.54254/2753-8818/21/20230860

4. Molecular chaperones and protein folding

By avoiding protein misfolding and aggregation, the class of proteins known as molecular chaperones contributes significantly to protein folding and the maintenance of cellular proteostasis [5]. These chaperones are able to identify misfolded or partially folded proteins and attach to them, promoting normal folding and preventing the creation of harmful protein aggregates [14]. Heat shock proteins (HSPs) and chaperonins are two types of molecular chaperones that are important for protein homeostasis and the cellular stress response [14].

The significance of molecular chaperones in preventing protein misfolding and related disorders is evident in their involvement in several neurodegenerative diseases. For instance, Alzheimer's and Parkinson's diseases are characterized by the accumulation of misfolded proteins in the brain, such as amyloid beta and alpha-synuclein, respectively. Studies have shown that overexpressing molecular chaperones can reduce protein aggregation and alleviate disease symptoms in animal models of these diseases [15]. Therefore, enhancing the function of molecular chaperones may represent a promising therapeutic approach for treating mental health disorders associated with protein misfolding.

Simply, molecular chaperones are essential proteins that facilitate proper protein folding and help maintain cellular proteostasis by preventing protein misfolding and aggregation. Their critical role in preventing protein misfolding and their potential as therapeutic targets for neurodegenerative diseases underscores the need to understand their mechanisms and develop strategies to enhance their activity.

5. ABeta plaques and tau tangles and mental disorder

The overlapping symptoms observed in major depressive disorder, bipolar disorder, and schizophrenia might be partially attributed to the presence of Aβ plaques and tau tangles, which are commonly associated with neurodegenerative diseases like Alzheimer's. These pathological features could be one of the key factors contributing to the development and manifestation of various mental health disorders.

Aβ plaques and tau tangles are known to interfere with neuronal function and communication, which may contribute to the observed similarities in the symptoms of these mental health disorders. These pathological features can disrupt various processes, such as synaptic transmission, neuroplasticity, and neurogenesis, that are essential for maintaining healthy brain function.

The presence of Aβ plaques and tau tangles can also lead to neuroinflammation and oxidative stress, which may further exacerbate the symptoms of these disorders. Neuroinflammation and oxidative stress have been implicated in the pathogenesis of several mental health disorders and could serve as a common denominator linking these conditions to Aβ plaques and tau tangles.

In addition, Aβ plaques and tau tangles may indirectly contribute to the observed symptom similarities by affecting other molecular pathways and cellular processes implicated in these disorders, such as the dysfunction of glutamatergic signaling, mitochondrial dysfunction, and altered expression of proteins involved in synaptic plasticity and neurotransmitter release.

5.1. MDD and the link to Aβ plaques and tau tangles

Merging evidence suggests that there may be a connection between depression and the presence of Aβ plaques and tau tangles, which are characteristic of Alzheimer's disease [16]. Although the exact mechanisms underlying this relationship remain unclear, several hypotheses have been proposed.

One possibility is that the accumulation of Aβ plaques and tau tangles may disrupt neuronal function and communication, leading to depressive symptoms. In some studies, higher levels of Aβ and tau have been observed in the brains of individuals with a history of depression, suggesting a potential link between these pathological features and the development of depressive symptoms.

Another hypothesis is that the chronic stress and inflammation associated with depression may contribute to the formation and accumulation of Aβ plaques and tau tangles. Prolonged stress and inflammation can impair the brain's ability to clear misfolded proteins, leading to their accumulation and the formation of toxic aggregates. This, in turn, may exacerbate depressive symptoms and increase the risk of developing neurodegenerative diseases such as Alzheimer's.

DOI: 10.54254/2753-8818/21/20230860

Moreover, alterations in neurotrophic factors, such as BDNF, have been implicated in both depression and Alzheimer's disease. As mentioned earlier, misfolded BDNF can impair neuronal growth and survival, which may contribute to depressive symptoms. Additionally, decreased BDNF levels have been linked to the progression of Alzheimer's disease, as they can exacerbate the accumulation of Aβ plaques and tau tangles, further impairing neuronal function.

In conclusion, there appears to be a complex interplay between depression, Aβ plaques, and tau tangles, with the potential for a bidirectional relationship between these factors. Understanding the precise mechanisms underlying this relationship may help to identify novel therapeutic targets and strategies for the treatment of depression and the prevention of neurodegenerative diseases such as Alzheimer's.

5.2. BD and the link to Aβ plaques and tau tangle

The potential involvement of Aβ plaques and tau tangles, characteristic of Alzheimer's disease, in the pathophysiology of bipolar disorder has attracted growing interest. The disorder is marked by episodes of mania and depression, with the underlying causes believed to arise from a combination of genetic and environmental factors.

Post-mortem studies of patients with bipolar disorder have shown an increased presence of protein aggregates in the brain, including amyloid-beta plaques and tau tangles [10]. These findings suggest that these pathological features may contribute to the development of bipolar disorder.

Altered expression of proteins involved in synaptic plasticity and neurotransmitter release, such as DISC1 and GSK-3β[1], has been observed in bipolar disorder. These proteins may also be linked to the formation and accumulation of Aβ plaques and tau tangles. Dysregulation of these proteins can disrupt neuronal function and communication, potentially exacerbating the symptoms of bipolar disorder and increasing the risk of developing neurodegenerative diseases such as Alzheimer's.

Mitochondrial dysfunction has been implicated in the pathogenesis of bipolar disorder [13]. Mitochondria play a crucial role in energy production and calcium signaling within neurons. Dysfunctional mitochondria can lead to an imbalance in calcium signaling and oxidative stress, which can contribute to neuronal damage and death. Research has shown that the accumulation of Aβ plaques and tau tangles can impair mitochondrial function, potentially contributing to the development of bipolar disorder [14].

5.3. SCZ and the link to Aβ plaques and tau tangles

Protein misfolding has been implicated in schizophrenia with evidence showing altered expression of proteins involved in synaptic transmission and neuroplasticity, such as neuregulin 1 (NRG1) and dysbindin (DTNBP1) [1]. Post-mortem studies have demonstrated an increased presence of protein aggregates in the brains of schizophrenia patients, including amyloid-beta plaques and hyperphosphorylated tau, suggesting a potential role of these pathological features in the pathophysiology of the disorder [12].

The dysfunction of glutamatergic signaling in the brain has been implicated in the pathophysiology of schizophrenia. Glutamate is a neurotransmitter that plays a key role in synaptic plasticity and cognitive function. Accumulation of Aβ plaques and tau tangles may interfere with the proper folding and trafficking of glutamate receptors, leading to altered glutamate signaling and synaptic dysfunction in schizophrenia.

Furthermore, the presence of Aβ plaques and tau tangles may contribute to neuroinflammation in schizophrenia. The accumulation of misfolded proteins in the extracellular space can activate the immune system and induce an inflammatory response in the brain. This neuroinflammation has been linked to the pathogenesis of schizophrenia and may contribute to the development of the disorder.

DOI: 10.54254/2753-8818/21/20230860

6. Current technical challenges in protein misfolding research

6.1. Identifying and characterizing misfolded proteins

One of the key challenges in protein misfolding research is the identification and characterization of the specific misfolded proteins and their conformations that contribute to mental health disorders. Characterizing the dynamic and heterogeneous nature of misfolded protein aggregates is difficult using conventional experimental methods like nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography [13].

6.2. Development of reliable and sensitive detection method

Detecting misfolded proteins and their aggregates in biological samples, particularly at early stages of aggregation, remains a technical challenge. Current methods, including immunoassays and fluorescence-based techniques, can be limited by low sensitivity and specificity, as well as high background noise [13].

6.3. In vivo modeling

Developing accurate and reliable animal models that recapitulate the complexity of human mental health disorders associated with protein misfolding is crucial for understanding disease mechanisms and testing potential therapies. However, creating such models is challenging due to species differences in protein sequences, folding pathways, and cellular environments [1].

6.4. Targeting protein aggregates

The development of therapeutic strategies that specifically target misfolded proteins or their aggregates without affecting the function of normally folded proteins is a significant challenge. Small molecules or biologics that selectively bind to misfolded proteins can sometimes have unintended off-target effects, and the blood-brain barrier can limit the delivery of potential therapeutics to the central nervous system [8].

6.5. Enhancing molecular chaperone function

While molecular chaperones represent a promising therapeutic approach, modulating their function without disrupting cellular homeostasis is challenging. Developing small molecules or other therapeutic agents that selectively enhance the activity of specific chaperones or chaperone networks without causing toxicity or off-target effects is a critical goal in this area [16].

7. Future directions in research

To create new therapeutic approaches, it is crucial to have a clearer understanding of the molecular processes that underlie protein misfolding in neurological illnesses. Current research is focused on identifying the specific protein misfolding events contributing to mental health disorders and developing novel therapeutic interventions targeting misfolded proteins [8]. In the future, it will be crucial to:

7.1. Develop new experimental and computational tools to better characterize misfolded proteins and their aggregation dynamics

This objective involves the creation and optimization of novel experimental techniques and computational methodologies to advance the understanding of misfolded proteins, their aggregation properties, and the factors that influence their formation and stability. This includes the development of high-resolution imaging techniques, single-molecule biophysical assays, and advanced computational modeling approaches to elucidate the molecular and structural basis of protein misfolding and aggregation.

DOI: 10.54254/2753-8818/21/20230860

7.2. Improve the sensitivity and specificity of detection methods for misfolded proteins and their aggregates in biological samples

Enhance existing detection methods or create new, innovative techniques for identifying and quantifying misfolded proteins and their aggregates in various biological samples, such as tissue, blood, or cerebrospinal fluid. This may involve the development of high-throughput screening assays, nanoscale imaging technologies, and biosensors with improved selectivity and sensitivity to detect misfolded proteins at early stages, enabling early diagnosis and intervention.

7.3. Design therapeutic strategies that selectively target misfolded proteins or their aggregates without affecting the function of normally folded proteins

Create innovative therapeutic approaches that specifically target and neutralize misfolded proteins or their aggregates while preserving the function of normally folded proteins. This may involve the development of small molecules, antibodies, or other biologics that selectively bind to misfolded proteins, as well as the design of strategies to modulate cellular pathways involved in protein folding, degradation, and clearance to prevent or reverse protein misfolding and aggregation.

7.4. Investigate the potential of molecular chaperones as therapeutic targets and develop approaches to selectively enhance their activity without causing toxicity or off-target effects

Explore the role of molecular chaperones, which assist in protein folding and quality control, as potential therapeutic targets in the context of protein misfolding disorders. Develop methods to selectively enhance the activity of specific chaperones, without causing adverse side effects or off-target consequences, in order to promote proper protein folding and prevent the formation of toxic protein aggregates. This may involve the design of pharmacological modulators, gene therapies, or other approaches to regulate chaperone activity and expression.

8. Conclusion

In conclusion, this study delved into the critical role of protein folding as a complex and essential biological process that ensures proper protein function. The investigation specifically explored the impact of protein misfolding, changes in amyloid-beta (Aβ) plaques, and tau tangles on neurological disorders, particularly depression, bipolar disorder, and schizophrenia. Misfolded proteins can become toxic, accumulating within cells and leading to a variety of neurological disorders that significantly affect an individual's mental well-being.

We also examined the role of molecular chaperones in preventing protein misfolding and their potential as therapeutic targets in treating mental health disorders. Our findings highlight the importance of molecular chaperones in maintaining cellular proteostasis and mitigating the formation of Aβ plaques and tau tangles, both of which contribute to the progression of neurological disorders.

To fully comprehend the specific mechanisms by which protein misfolding, Aβ plaques, and tau tangles contribute to mental health disorders, further research is necessary. Scientists must dive deeper into the complex interactions between proteins, molecular chaperones, Aβ plaques, and tau tangles to identify innovative therapeutic interventions targeting these elements. Developing new treatments that address protein misfolding, Aβ plaque formation, and tau tangle accumulation could help alleviate the symptoms of mental health disorders and improve the quality of life for those affected by these debilitating conditions.

By integrating the consequences of protein misfolding, alterations in Aβ plaques, and tau tangles, along with the preventive role of molecular chaperones, this study offers a comprehensive perspective on the potential therapeutic avenues for treating neurological disorders associated with depression, bipolar disorder, and schizophrenia.

DOI: 10.54254/2753-8818/21/20230860

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230861

Beyond smallpox: The emergence of monkeypox as a threat to human health - symptoms and treatment

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Abstract. A double-stranded DNA pox virus known as monkeypox is a member of the *Poxviridae* family, which is divided into the West African and Congo Basin clades. Spreading originally from countries in Africa. Monkeypox has since spread to numerous other countries and was proclaimed a worldwide health emergency. There are no current treatments designed for monkeypox, therefore it is important to discuss the therapeutic options and prevention methods. For the symptomatic management of monkeypox, antiviral medications are utilized. Numerous studies conducted in animal models proved the safety and efficiency of antiviral drugs designed for monkeypox, despite the risk factors. The monkeypox virus can also be prevented by using previous smallpox vaccines. Here, we focus on the epidemiology, the transmission and the pathology of monkeypox. Further, we review the current therapies and vaccines for monkeypox to help to limit the spread and influence of monkeypox, and further, to bring insight in further studies involving antiviral drugs and vaccines to treat monkeypox.

Keywords: Monkeypox, Orthopoxvirus, Antiviral Drugs, Vaccine.

1. Introduction

Since the emergence and eradication of smallpox, a new and closely related zoonotic disease, the monkeypox virus, has appeared as a rare but impactful global outbreak. While monkeypox is a milder variation of smallpox, it has identical clinical manifestations and transmission mechanisms. Usually, infecting rodents or nonhuman primates and rarely spreading to people, monkeypox is a rare condition brought on by the pox virus from the *Poxviridae* family [1, 2]. Despite their inability for mRNA translation, their distinctive structure and DNA enable replication and transcription assembly with their own genomes. It is believed that primates such as squirrels, rats, monkeys, and primates are the main carriers of monkeypox in the African regions [1]. Although the Democratic Republic of the Congo saw the first human instance of monkeypox in 1970, it has since been linked to numerous additional human cases in Africa as well [1]. With the initial spread of monkeypox to other countries, many countries faced a shortage of vaccines and antiviral drugs. Since then, vaccines and antiviral drugs for monkeypox are now available for providers and patients. There are currently cases of monkeypox in numerous nations, including the United States, largely supported by a lack of prior smallpox vaccinations [1]. Particularly in the DRC and Central and West Africa, the monkeypox virus remains a severe hazard to human life. Human interactions with one another through respiratory droplets or close

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DOI: 10.54254/2753-8818/21/20230861

exposures to an infected person or their fluids is the main cause of the current outbreak. Due to the similarities between monkeypox and smallpox, clinical differentiation between the two is also challenging unless with specific tests [1]. With the ongoing outbreak of monkeypox, there are increasing concerns of monkeypox becoming a more severe human pathogen, bringing in more waves of the outbreak. Currently tests of antiviral drugs and vaccines are now being conducted for their effectiveness against monkeypox. This review will discuss the definition and pathology of monkeypox and the current possible treatment options for monkeypox. It will also discuss the current trials and studies done with antiviral drugs and the two vaccines specified for monkeypox.

2. Epidemiology of monkeypox

2.1. Epidemiology

In Copenhagen at the end of 1958, the monkeypox virus was first identified. It was given the name "monkeypox" both for its resemblance to other poxviruses and the cynomolgus monkeys that it afflicted [1]. The first instance of monkeypox in a human was discovered in Africa in 1970 [1]. From 1970 to 1971, six cases of human monkeypox were detected, most of which were young children without the injection of smallpox virus [1]. Thereafter, the monkeypox virus causes many outbreaks in African nations between 1970 and 1986, with most cases occurring in central Africa, especially in the DRC, and a small number of cases in western Africa [3]. During these years, several Congo Basin countries include Cameroon, Central Africa Republic, and DRC report 394 cases of monkeypox in total [3]. Monkeypox outbreaks have been predominant outside of Africa since 2003, alongside the very initial case being detected in central Western America [2]. The following crucial outbreak of monkeypox swept through Nigeria in the period between 2017 and 2018 with a mortality rate of 6% [4]. Reported cases are 122, in which contains seven death cases, indicating a mortality rate of 6% [4]. 10 cases are reported that they have direct contact with animals [4]. After this outbreak, it is thought that the virus is transmitting through a new way, as it appears in city environment and the patients sometimes have genital lesions, which confirms that sexual contact may be a means of virus transmission [4]. At the year of 2022, there is another monkeypox outbreak in European countries and in North America. Portugal, Spain and Canada each reports cases of 14, 7, and 13 [4]. In 2022 May 20th, Netherland, Germany and France also report their first case. Another 11 instances were reported on the same day by the UK's health secretary, raising the total number of cases in excess of 70 [4]. Notably in this outbreak, most incidents occur between men and men with a sexual relationship. These cases are increasing rapidly mostly through sexual contact, with a chance that the virus might also spread through the air.

2.2. Monkeypox clades

The Western Africa Clade and the Central Africa, or Congo Basin, Clade are two distinct subgroups of monkeypox. Studies suggest that the sequence similarity between two clades of monkeypox is around 95% or over 99% [5]. The Congo Basin Clade is more harmful and deadly, and with an average percentage of deaths of 10.6%, the clade can transmit more easily from one individual to another. [5]. The Central Africa Clade is detected in the cases found in DRC and South Sudan [5]. It is thought that the Central African Clade is associated with milder symptoms, even though the human-to-human transmission is not readily obvious, and it has a much lower mortality rate of 3.6% [5]. It is believed that cases in Nigeria from 2010 to 2019 are infected by the Western Africa Clade [5]. The clade is also responsible for all cases that happened outside of Africa, including the current monkeypox outbreak [5]. Unexpectedly, it is discovered that there are 40 mutations in all among the isolates of the present monkeypox outbreak, most likely as a result of its capacity for human-to-human transmission [6].

2.3. Transmission and symptoms of monkeypox

The animal host of monkeypox virus remains unclear, but the major animal infection sources are identified as rodent animals, especially infected prairie dogs, which are responsible for the 2003

DOI: 10.54254/2753-8818/21/20230861

outbreak of monkeypox in US [1]. Transmission from patient to patient typically occurs when contaminated skin, body fluids, or respiratory droplets come into connection with each other [4]. Monkeypox virus could also have Mother-to-child transmission (MTCT) through placenta and lead to congenital monkeypox infection [6]. The virus enters the body, multiplies swiftly where it was introduced, and then quickly spreads to surrounding lymph nodes, which will cause lymphadenopathy which is a specific symptom observed on monkeypox patients [6]. According to clinical diagnosis, monkeypox virus takes up to 21 days for developing an explicit symptom, usually accompanied by some prodromal illness including headache, fever, and lymphadenopathy [6]. Within up to 10 days of the virus developing, the exanthema stage, which follows the prodromal stage, is accompanied by vesiculopustular rashes that first appear on the face and then spread throughout the body [6]. The virus is self-limiting and after 2 weeks of exposure, an antigen could be detected in the serum [6]. Many patients in the most recent monkeypox outbreak in 2022 displayed vaginal and peri-anal infections, swelling of the lymph nodes, fever, and difficulty swallowing, whereas some of the patients merely displayed symptoms in the form of a few sores [7]. Meanwhile, though not very clear, the route of infection might also influence the clinical symptoms [8]. Patients with respiratory or mucosal infection experienced a more typical monkeypox illness progression, including a 2–3-day febrile prodrome, less overall systemic symptoms, and no accompanied gastrointestinal involvement [8].

3. Pathogenesis of monkeypox

Monkeypox is a kind of *Orthopoxvirus*. These viruses have a genomic size of 130 to 360 kbp and are large, linear, double-stranded DNA viruses [1]. Genes important in crucial processes like transcription and virus assembly are found in the genome's center, whereas those near the termini are engaged in interactions between viruses and their hosts, such as host range restriction and immune evasion [1]. Due to the large sizes of poxviruses, they are much harder to breakthrough the immune defense of the host [2]. Thus, as a response to evade from the host's immune system, the virulence genes of orthopoxviruses produce a series of chemicals that will serve as modulators by targeting elements of the host's immune system [1]. There are mainly two groups of modulator proteins as shown in the graph (Figure 1). In the case of intracellular modulatory proteins, virotransducer proteins will function through interfering cells' response ability to extracellular virus invasions, including the oxidative burst and apoptosis mechanism [2]. Virostealth proteins function inside the cell as well. To reduce the chance that the host's immune system would detect the virus, they work by downregulating immune recognition markers, such as MHC I and $CD4^+$ [1, 2]. Extracellular modulatory proteins also play an important role in poxvirus infection. The viroreceptors, a type of glycoprotein, competitively bind the cytokines and chemokines of the host, preventing them from fulfilling their intended function [2]. The viromimic proteins will modulate immune system response. Virolokines are created to limit host responses that are harmful to viral life, boosting responses that are crucial for viral reproduction and dissemination.

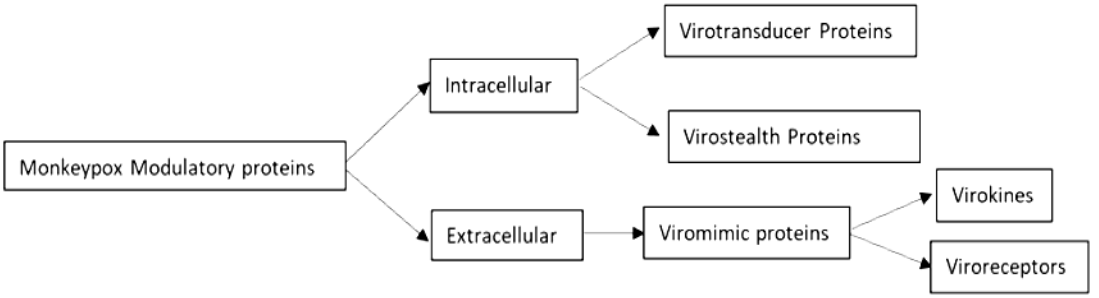

Figure 2. Types of monkeypox modulatory proteins.

DOI: 10.54254/2753-8818/21/20230861

In the case of monkeypox, DNA replication happens in cytoplasmic structures which are referred to as factories [9]. Factories are DNA structures that are compressed in the early stages of infection and are enclosed with membranes that appear to come from the cell's rough endoplasmic reticulum [9]. Every factory is the product of a single contagious particle [2]. These factories will keep enlarging with the continuous formation of DNA. A more erratic shape will also eventually take shape when cavities containing viral mRNA and host translation factors form [1, 9]. When the replication cycle is about to finish, the endoplasmic reticulum membranes that surround the cell break down and produce crescent-like structures that serve as the building blocks for the immature virions (IV). A collection of viral membrane building proteins as well as a complex of late gene products will be generated [1]. Then, from the IV, the most prominent infectious species, MV, are produced [9]. Following their incorporation with the cytoplasmic membrane, these MV will leave the cell [2].

4. Clinical therapy of monkeypox

Presently, no identified treatments are available for monkeypox. Despite this, antiviral drugs such as cidofovir, brincidofovir, and tecovirimat, based on previous smallpox experiences, may be helpful in the treatment of monkeypox [10]. By preventing viral DNA polymerase from functioning, these antiviral medications primarily stop the spread of both smallpox and monkeypox. Several of these antiretroviral studies have been carried out in humans, but there is still little proof of their effectiveness [4]. The antiviral drug, cidofovir, could potentially work, however, it has not been recognized by the FDA for treating monkeypox and should only be utilized in extreme situations under proper authority. The specific advantages of cidofovir are still unknown and can have negative effects, despite research in vitro and on animals showing beneficial results [7]. The nucleotide analog of cidofovir, brincidofovir, is a lipid conjugate of cidofovir. In comparison with cidofovir, brincidofovir has faster enzyme conversion to its active form, has better cellular uptake, and is also orally bioavailable, being able to be created into tablets for drug delivery. As a result, the use of brincidofovir may be safer than cidofovir. Additionally, brincidofovir has demonstrated encouraging outcomes in numerous *Orthopoxvirus* animal models, particularly in a prairie dog model where it increases the survival rates against monkeypox [10]. However, regarding the use of brincidofovir for monkeypox, it was briefly discontinued due to the possibility of hepatic dysfunction [7]. Currently, not enough data is present to prove the efficacy of brincidofovir in humans but based on the studies conducted in animal models, brincidofovir could be used to reduce symptoms of monkeypox while carrying minimal risks. Another antiviral medication, tecovirimat, or TPOXX, ST-246, has demonstrated effective results in treating diagnosed instances of monkeypox. Being first approved for smallpox treatment, tecovirimat can stop the spread of monkeypox by blocking the VP37 protein on the virus' envelope [10]. The use of tecovirimat for diseases brought on by orthopoxviruses, such as monkeypox, has been authorized by the CDC [7, 10]. Results from clinical trials have proven the safety of using tecovirimat on humans and show improved survivability rates against lethal monkeypox strains. The results showed that when injected with a dose of tecovirimat for up to 5 days before the challenge of monkeypox, the antiviral drug can reduce the amount of virus in the body, suppressing many of the symptoms, and reduces the spread of monkeypox [10]. Having undergone testing in both human and animal models, with positive outcomes, the FDA has approved tecovirimat for clinical applications [7]. It can be taken orally as an oral capsule or injected intravenously. However, tecovirimat is difficult to obtain for less severe cases of monkeypox because of its limited supply. Although promising results have surfaced from each of the antiviral therapeutic drugs, currently, more insight is necessary to accurately evaluate the safeness and direct causes of each antiviral medicine.

4.1. Vaccines

Besides taking antiviral drugs as a treatment, vaccines are also repurposed for monkeypox. The characteristic of the vaccine allows the body to stimulate its protective immune responses without the virus infecting the body or spreading. According to data, several smallpox vaccines have demonstrated similar effects against the monkeypox virus and can be used as a preventative against the disease [10].

DOI: 10.54254/2753-8818/21/20230861

Today, JYNNEOS and ACAM2000, are the two main vaccines used for the prevention of monkeypox, exhibiting comparable protection against smallpox and monkeypox [10]. The JYNNEOS vaccine is produced by altering the attenuated, non-replicating Ankara-Bavarian Nordic variant from the *Orthopoxvirus*. The FDA authorized and cleared JYNNEOS for use in adults as a smallpox and monkeypox disease preventative [10]. In studies conducted in non-human primates, the effectiveness of JYNNEOS provided complete protection against a lethal monkeypox threat. In clinical studies regarding the use of JYNNEOS in animal models, vaccinated animal models expressed less mortality rate when compared to unvaccinated animal models [11]. These research and data have made JYNNEOS the preferred vaccination for monkeypox due to its effectiveness in protecting against smallpox and monkeypox without major adverse effects. However, JYNNEOS is only available to adults who have been found to have a hazard risk of developing fatal symptoms. In addition, the FDA has also authorized the ACAM2000 vaccine to treat monkeypox during an outbreak. Similar to JYNNEOS, the ACAM2000 vaccine contains the live vaccinia virus. Unlike JYNNEOS, the vaccinia virus in ACAM2000 is capable of replication, infecting cells, and producing infectious particles. As a result, using ACAM2000 carries a danger of inadvertent inoculation and autoinoculation, however, using

Table 1. Comparison between ACAM2000 and JYNNEOS.

Characteristics	**Name**	
	ACAM2000	**JYNNEOS**
Vaccine type	Self-Replicating	Unable to replicate itself
Recommended to take vaccine	Yes	Yes
Possible adverse events	Yes	No major risks proven with tests
Risk of cardiac events	5.7 cases per 1,000 people	Not enough clinical tests to accurately determine risks
Effectiveness	Insufficient clinical result to determine efficacy against monkeypox	Can reduce risk and transmission of monkeypox
Administration	Single dose	Consists of 2 doses, about 4 weeks separate

JYNNEOS carries no such risk (Table 1) [10]. Studies employing ACAM2000 on animal models showed that the survival rate was higher than that of unvaccinated animal models [11]. Nevertheless, statistics indicated that JYNNEOS provided a greater survival rate when compared to ACAM2000. Receiving JYNNEOS is recommended for adults with high risk of experiencing serious side events from ACAM2000 and those who have severe monkeypox disease. Studies employing ACAM2000 on animal models showed that the survival rate was higher than that of unvaccinated animal models [11]. Additionally, it should be highlighted that immunizations are exclusively to prevent monkeypox. Other than the use of antiviral medications to lessen some of the symptoms, there are no specific treatments after the initial infection of monkeypox.

5. Conclusion

The spread of monkeypox among people around the world has raised concerns and put many families' lives in danger. Since its first discovery in the continent of Africa, monkeypox has spread across the world, creating a multi-country outbreak. Initial outbreaks of monkeypox caused shortages of treatments across the world. Over the years, with research and development in technology, recent studies figured out the transmission and pathology of monkeypox. Using pre-existing therapeutic drugs and vaccines of smallpox, which shows the similar feature as monkeypox, people with monkeypox were treated from their complications. Using non-human primate and animal models, those antiviral drugs and vaccines were tested for their effectiveness. Despite lacking clinical trials and

a defined treatment for monkeypox, emerging studies of antiviral drugs and smallpox vaccines have shown effective results to combat cases of monkeypox. The safety and accessibility of the present antiviral medications and vaccines would increase with further clinical trials and testing. With continued development of antiviral drugs and vaccines, the therapy of monkeypox could be more effectively. However, despite ongoing outbreaks across the world, awareness and knowledge of monkeypox remains uncommon among the population. In addition, hesitancy towards vaccination is also common among the population. Future efforts to combat monkeypox and other illnesses may be more coordinated if there is greater understanding of monkeypox and vaccine acceptance. Although it may not be possible to eradicate monkeypox in the foreseeable future, with developing treatments and therapeutics, severe symptoms of monkeypox can become less lethal over time.

Authors contribution

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

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DOI: 10.54254/2753-8818/21/20230861

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230863

Comparative analysis of efficacy and safety of ChAdOx1 nCov-19 and AD26.COV2.S

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Abstract. Vaccine technology has been going through some radical and incremental advancement over the years for its importance as the most crucial tool in the prevention and elimination of transmissive diseases. Since the outbreak of COVID-19, several vaccine platforms were being studied, developed, and entered the market. Among the platforms, vector viral vaccines attracted particular attention for the cooperation of viral vectors to deliver genetic material of the pathogens. While the mechanism has improved the flexibility of vaccine design and efficacy, the adverse events after vaccination were worth attention at the same time. Taking ChAdOx12 nCov-19 and AD26.COV2.S. as the subjects, the efficacy and safety of the two vaccine platforms were compared and analyzed. In this paper, the initial vaccine efficacy, the resistances against virus variants, and various post vaccination adverse events were looked in more detail to identify the different factors that could affect the performance of viral vector vaccines.

Keywords: vaccine, efficacy, adverse events, safety.

1. Introduction

The outbreak of COVID-19 has been drawing increasing attention to idea of vaccination and development of vaccines. Within one year after the case was discovered, the first COVID-19 vaccine was successfully developed and implemented. Since end of 2020, two viral vector vaccines were under the research and development - ChAdOx12 nCov-19 developed in the UK and AD26.COV2.S developed in the US. The efficacy of the two vaccines were proven to be adequate and the implementation was sped up due to the emergency. However, in April of the same year, the US and several other countries suspended the use of ChAdOx12 nCov-19 due to the concern about blood clots. The incidence was also discovered in AD26.COV2.S. The data was collected from the studies from independent research papers and the comprehensive reports published by US FDA and EMA. The latter had a more comprehensive systematic evaluation of vaccine efficacy and safety; therefore, it is difficult to integrate the results. With the absence of analysis of certain aspects, only a general comparison could be made. Although a clear view of how the two types of vaccine were different in terms of efficacy and safety, a deeper analysis of why they varied in these two areas still needs to be carried out. The efficacy of the two vaccines was compared with consideration of the population characteristics of the test subjects and their performance against different types of virus variants, and the safety analysis focused on the local adverse events and rare adverse events VITT/TTS. The

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DOI: 10.54254/2753-8818/21/20230863

purpose is to distinguish the factors that may affect the performance of the vaccines with the same vaccine platform.

2. Comparison of safety and efficacy of ChAdOx12 and AD26.COV2.S

2.1. ChAdOx12

ChAdOx12 was together developed by Oxford University and AstraZeneca and was first licensed in December 2020 in UK. To achieve the desired efficacy, the vaccination facility requires a total of at least two doses to be injected into the deltoid muscle by intramuscular injection at intervals of 4 to 12 weeks. It utilized ChAdOx1 nCov-19, a type of cold-causing chimpanzee adenovirus based on simian adenovirus type Y25 as the vector with COVID-19 virus spike protein-producing gene inserted to produce the antigen spike protein that induces human immune responses and the tissue plasminogen leader sequence which was used to enhance immunogenicity and expression of the protein [1]. The chimpanzee adenovirus type Y25 was chosen to avoid pre-existing immunity to common human adenovirus from vaccines for its unique feature to have chimpanzees as the only natural host. The spike protein-producing gene was modified to increase protein production; the vector has E1 and E3 genes deleted to deprive the vector of replication ability, E4 genes modified to carry human Adenovirus serotype 5 genes for better insertion capability and bettered manufacturing process [1]. After injection, ChAdOx12 nCov-19 can induce strong, durable immune responses inside the human body and offers protection from severe and moderate SARS-CoV-2 symptoms.

2.2. AD26.COV2.S

AD26.COV2.S was under development by the American multinational pharmaceutical cooperation several months after ChAdOx12 nCov-19 was licensed and was licensed in February 2021. The two had the same type of injection. Different from ChAdOx12 nCov-19, only one dose was required by the official. Replication-deficient human adenovirus was chosen to be the vector that carries gene encoding prefusion-stabilized spike protein. The spike protein is stabilized by six prolines proteins making itself a more effective variant in stimulating the creation of antibodies [2].

2.3. Comparison of efficacy

When comparing different types of vaccines, one cannot ignore various factors that can have prominent effects when studying the efficacies of the vaccines, including methodology, population characteristics, and variants of the virus. Specifically in population characteristics, key factors such as age, comorbidity, and severity of symptoms need to be analyzed separately.

2.3.1. Population characteristics. Phase III trials for COVID-19 vaccines were done with populations with different characteristics. A total of four trials in different countries for ChAdOx12 nCov-19 and one trial for AD26.COV2.S were conducted. Study subjects from several countries were selected for the two vaccines, four trials were conducted for ChAdOx12 nCov-19 by experts from several institutes from UK, and two trials for AD26.COV2.S were conducted by US FDA and EMA respectively. All study subjects were adults aged over 18 years. The method of the experiment may have a considerable impact on the efficacy result of the vaccine. Characteristics of which the method designed for the experiment include age, sex, race, ethnicity, country of the study subjects, the period of follow-up investigation and the definition of a main endpoint of each trial are essential factors for the final number. The following Table 1 shows the basic information about the population characteristics of the trials.

DOI: 10.54254/2753-8818/21/20230863

Table 1. Phase III trials for ChAdOx12 nCov-19 and AD26.COV2.S [3].

Vaccine Brand	Subject of Study	Experiment Target
AZD 1222	United Kingdom	Symptomatic cases, more than 14 days after the final dose with no history of infection
	United Kingdom, Brazil, and South Africa	Symptomatic cases, more than 14 days after the final dose with no history of infection
	South Africa	Mild to moderate cases, more than 14 days after the final dose without prior infection
	United States	Prevention of symptomatic cases
AD26.COV2.S	United States, Mexico, South American countries, and South Africa	Moderate to critical cases, more than 14 and 28 days after vaccination with no history of infection

2.3.1.1. ChAdOx12 nCov-19. For the first trial in the table, the participants were recruited in the UK during second half of 2020 and received booster doses during the same time of the year. Among 8534 participants, 78% (6636) were aged 18-55 years and 59% (5065) were females [4]. 520 of them developed SARS-CoV-2 infections between Oct 1, 2020, and Jan 14, 2021. It was observed that the neutralization level of antibodies induced by the vaccine against B.1.1.7 was lower than Victoria lineage. The clinical vaccine efficacy for B.1.1.17 was 70.4% and for non-B.1.1.7 was 81.5% [4].

For the second trial, the participants were recruited from three different countries – Brazil, South Africa, and the UK. The diversity of study subjects was much higher in terms of race where white, black, Asian and other races were all included. The population base of study subjects in the trial was much bigger in addition. During the time between April and November of 2020, a total of 23,848 participants were introduced into the trial and were vaccinated across four studies including a total of 11,750 participants from the United Kingdom, 10,002 participants from Brazil, and 2,096 from South Africa [5]. Half of the participants who met the criteria received 2 doses of ChAdOx12 nCov-19 and another half received the same number of doses of the placebo. The average vaccine efficacy for recipients in the UK was 73.5%, and that for Brazil was 64.2%. Among the UK recipients, those who had a lower first dose showed a much higher vaccine efficacy [5]. The difference between the vaccine efficacy from the UK and Brazil might be due to the different virus variants that were prevalent in these two countries.

The third trial focused specifically on B.1.351. which was the variant discovered in South Africa. Between Jun 24 and Nov 9, 2020, 2,026 adult participants from South Africa were recruited in which half of them received single dose of the vaccine and another the placebo. In the laboratory neutralization process, greater resistance to B.1.351 was shown. In addition, 39 out of 42 participants who developed Covid-19 had this variant. The vaccine efficacy against the variant was only 10.4% [6].

The last ChAdOx12 nCov-19 trial was conducted in the US. The trial showed 79% efficacy in the prevention of the symptomatic cases and 100% in prevention of severe cases that required hospitalization [7]. The trial had 32,499 participants with various ages and ethnic identities. Although it was said to have a consistent vaccine efficacy across these identities, a notably higher efficacy of 80% was observed in the participants aged 65 years and older [7].

2.3.1.2. AD26.COV2.S. US FDA has analyzed the efficacy of AD26.COV2.S in several different subgroups. In each subgroup, more than 14 or 28 days after the vaccination was separately analyzed. As indicated in the tables shown below, the factors such as comorbidity, age, severity of symptoms and country of participants could greatly affect the efficacy of AD26.COV2.S. The vaccine efficacies with consideration of age, comorbidity, the severity of symptoms, region of the subjects and source of confirmation were shown in Tables 2-4 below. All data of vaccine efficacy refers to the condition of

DOI: 10.54254/2753-8818/21/20230863

the first occurrence, including both centrally confirmed and non-centrally confirmed cases except for the Table 3.

Table 2. Moderate to critical cases [8].

Subgroup/Days After Vaccination	≥ 14	≥ 28
Comorbidity		
Yes	64.2%	58.6%
No	67.6%	68.8%
Age group and comorbidity		
18-59, no	65.6%	68.0%
18-59, yes	63.9%	64.0%
≥ 60, no	76.0%	72.4%
≥ 60, yes	64.0%	42.3%

Table 3. Against adjudicated severe/critical cases [8].

Days After Vaccination	≥ 14	≥ 28
Centrally confirmed cases		
Overall	76.7%	84.5%
18-59 years	80.5%	91.7%
≥ 60	68.8%	70.3%
Non-Centrally confirmed cases		
Overall	76.3%	83.5%
18-59 years	76.9%	85.0%
≥ 60	75.1%	80.2%

Table.4. Moderate to critical by country of participation [8].

Country/Days After Vaccination	≥ 14	≥ 28
United States		
Moderate to Severe/Critical	74.4%	72.0%
Severe/Critical	78.0%	85.9%
South Africa		
Moderate to Severe/Critical	52.0%	64.0%
Severe/Critical	73.1%	81.7%
Brazil		
Moderate to Severe/Critical	66.2%	68.1%
Severe/Critical	81.9%	87.6%

2.3.2. Variants of virus. Several variants of the COVID-19 virus are identified in different region of the globe, characterized by specific mutations in the virus that affect their transmissibility, the severity of symptoms and potential resistance to the existing vaccines. Table 5 below shows the effect of new variants on ChAdOx12 nCov-19 and AD26.COV2.S compared with the original virus.

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Table 5. Effect of variants on efficacy [3].

Variants (WHO nomenclature)	First Detection	Transmission and Morality	ChAdOx12 nCov-19	AD26.COV2.S
B.1.1.7	United Kingdom	+ 56% (UK) + 61-64% (UK)	2.1-fold reduction	NA
B.1.551	South Africa	+ 50% (South Africa) NA	3.3-23.45-fold reduction	NA
B.1.1.28.1.P1	Brazil and Japan	+160% (Brazil) NA	9-fold reduction	3.4-fold reduction
B.1.617.2 (and AY sublineages)	India	+ 40-60% (UK) More severe cases than Alpha	3.1-9-fold reduction	1.6-5.4-fold reduction
B.1.617.1	India	NA NA	1-2.6-fold reduction	NA

2.3.3. General comparison. Comparing the data from the vaccine efficacy studies of the two vaccines, the overall performance of ChAdOx12 nCov-19 was better than AD26.COV2.S. However, there is also a significant lack of more detailed analysis from ChAdOx12 nCov-19. On the other hand, ChAdOx12 nCov-19 is more suspectable to different types of variants. Therefore, when comparing the efficacy of the two vaccines, a more specific context is needed. Other reasons why ChAdOx12 nCov-19 had a better efficacy may be due to, first, the formulation of the vaccine where ChAdOx12 nCov-19 utilized chimpanzee adenovirus based on simian adenovirus type Y25 to avoid possible pre-existing immunity.

2.4. Comparison of safety and risks

The safety issues concerning the vaccines ChAdOx12 and AD26.COV2.S are the adverse event following immunization (AEFI). Common post-vaccination adverse events are associated with either local event that occurs near the site of injection or systemic unwell feelings including fatigue, headache, myalgia, etc. that can usually be dealt with regular oral over-the-counter medications with no need for hospitalization. Occasionally, a rare adverse event with a higher grade of severeness was found. Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) was one major rare severe adverse event that was observed in individuals vaccinated with adenovirus vector vaccines, thus including both ChAdOx12 nCov-19 and AD26.COV2.S vaccinated patients. It is a blood coagulation condition accompanied by a low count of platelets.

2.4.1. Incidence of local adverse event. The incidences of a local adverse event, injection-site pain in patients vaccinated with both types of vaccines showed a near 10% difference – 39.24% and 48.6% for ChAdOx12 nCov-19 and AD26.COV2.S vaccinated patients respectively. This may be because ChAdOx12 nCov-19 carries a higher dose of the viral vectors than AD26.COV2.S, and which subsequently may result in a severer inflammation response and thus the pain at the injection site. In addition, the time interval during the doses may also contribute to the injection site pain for the longer the interval, the more robust the immune response. Since ChAdOx12 nCov-19 officially requires two doses for a complete vaccination, the incidence of injection site pain was thus higher.

2.4.2. Incidence of general adverse event. Four main general adverse events were discovered and reported in a higher proportion of participants in the adverse event study, including headache, fatigue, myalgia, and nausea. In ChAdOx12 nCov-19 vaccinated participants, the respective percentages of participants who had been reported to have the four systemic adverse events were 36.20% (headache),

DOI: 10.54254/2753-8818/21/20230863

42.53% (fatigue), 43.04% (myalgia) and 3.54% (nausea) [9]. For AD26.COV2.S vaccinated participants, the percentages are 38.9%, 38.2%, 33.2% and 14.2% [10]. In comparison, higher incidences of fatigue and myalgia were observed in ChAdOx12 nCov-19 vaccinated participants and higher incidences of headache and nausea were observed in AD26.-=COV2.S vaccinated participants. The greatest differences occurred between the incidences of myalgia and nausea where 9.84% and 10.66% differences were observed respectively.

The myalgia observed in participants vaccinated with ChAdOx12 nCov-19 was often together with the symptom of polyarthralgia. The syndrome was acute and could persist for up to 47 days in severe cases. From experimental findings, the syndrome was likely to be caused by adenovirus vector-induced cytokine activation. Mouse model was used to conduct the experiment. The corporation of adenoviral vector has stimulated the innate immune system which subsequently led to an acute inflammation response. The response was accompanied with the secretion of cytokine. The impact of adenoviral vectors on vaccinated individuals depended on their age. For adults below 50 years old, the incidence of cerebral venous sinus thrombosis was often observed. The potential cause, platelet factor 4 – heparin antibodies were unexpected influential in a negative way. The symptom of myalgia is in addition coincidence with that of VITT which was one major identified rare adverse effect of adenovirus vector vaccines that had a higher incidence rate, especially in ChAdOx12 nCov-19 vaccinated individuals. The increase in cytokine secretion may cause and worsen the thrombosis by its ability to recruit leukocytes, the target of activated platelets [11].

2.4.3. Incidence of VITT. Since February 2021, there have been a few cases of uncommon but critical adverse reactions to vaccine-induced immune thrombotic observed in ChAdOx12 and AD26.COV2.S vaccinated individuals in several countries. Within these vaccinated individuals, a higher incidence rate of VITT was discovered in individuals vaccinated with ChAdOx12 nCov-19. In the USA, the rate of VITT was 3.55 x 10-6 % for those who were vaccinated with AD26.COV2.S, in comparison to 2 x 10-5 % to 1 x 10-6 % for those who received with ChAdOx12 in the United Kingdom. In Germany in which both types of vaccines were implemented into the medical system, 5.6 x 10-6 %were for AD26.COV2.S vaccinated people and 1.49 x 10-5 % for ChAdOx12 nCov-19 vaccinated people were reported [12].

The experiment utilized several experimental assays including sodium dodecyl sulfate gel electrophoresis, mass spectrometry, western blot analysis, proteasome activity assays, dynamic light scattering, zeta potential, zebrafish vascular permeability assay and 2 different types of microscopies, immunoelectron and transmission electron microscopy and super-resolution single-molecule light microscopy. Three major results were shown after the experiment.

First, the entire concentration of protein in the ChAdOx12 nCov-19 vaccine sample (102 ng/ L) was roughly 3.4 folds greater than the protein concentration in AD26.COV2.S vaccine sample (29.8 ng/ L). Second, a more complicated protein pattern for pure virus particles for ChAdOx12 compared with AD26.COV2.S sample was displayed by the gel electrophoresis. Third, a higher proportion and number of proteins were identified in ChAdOx12 nCov-19 vaccine sample (44.5% to 59.2%, 1571 31) by mass spectrometric analysis compared with AD26.COV2.S sample (0.26% to 0.96%, 59 14) [12].

One possible reason why the incidence of VITT was higher in ChAdOx12 nCov-19 than AD26.COV2.S was because the host cell protein impurity of ChAdOx12 nCov-19 was higher. Furthermore, platelet factor 4 (PF4) is the key protein identified in vaccine samples that causes VITT. It induces the clustering of vaccine components by forming a charge-dependent complex that contains hexon protein with its high affinity to the hexon protein and then mediates platelet-activating anti-PF4 antibodies that lead to VITT. In addition, EDTA-induced capillary leakage that occurred after the intramuscular injection of ChAdOx12 nCov-19 may also cause the difference in the rate of incidence of VITT.

DOI: 10.54254/2753-8818/21/20230863

3. Conclusions

By analyzing and comparing ChAdOx12 and AD26.COV2.S, the two vaccines from different companies with the same vaccine platform, it is evident that although the former had a higher efficacy performance overall, it had indicated a higher risk of adverse events after vaccination. Factors that may have influenced the result, including the characteristics of study population and experiment settings, are discussed in the analysis of vaccine efficacy in this paper. In addition, when comparing the efficacies of the two vaccines, patients with different conditions were studied, focusing on their age, comorbidities, the severity of their condition, and how their condition was identified. The two vaccines had different level of resistance when it comes to various variants of the Covid-19 virus. Form the statistics of the studies, it appeared that AD26.COV2.S was less affected by the variants. When analyzing the safety of the vaccines, both local and systemic adverse events were studied, and the results showed that both vaccines were involved into in a rare and potentially severe adverse event VITT/TTS. The ChAdOx12 nCov-19 tended to have a higher incidence rate of VITT/TTS than AD26.COV2.S due the presence of its complex protein composition. The mechanism behind the adverse event might also contribute to higher rate of certain local adverse events of ChAdOx12 nCov-19. Because the inconsistencies between the data obtained from the studies done to the two vaccines, some other aspects of comparison might be neglected. When more specific mechanisms behind the vaccines are discovered, there will be a step further digging into the causes of their differences.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230865

Mechanism and treatment of Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is a type of neurodegenerative disease that affects the nerves and tends to develop slowly, with symptoms worsening over time. It is responsible for causing 60 to 70% of cases of dementia, and it is becoming increasingly common. Currently, around 50 million people worldwide are affected, with the majority being over the age of 65. Despite the many experimental studies and cases, no treatment has been found that can prevent or reverse the progression of AD, and only a few methods are available that can temporarily alleviate symptoms. At present, the main focus of treatment is still on managing symptoms, although efforts are being made to reduce the production and impact of brain pathology. This article provides an overview of AD and its underlying causes, as well as current treatment options and possible future treatments that are undergoing testing. This article also discusses potential directions and predictions for new treatments that may be effective against AD.

Keywords: AD, pathogenesis, treatment.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and is often accompanied by many symptoms including deficits of new memory storage in the initial stages and a progressive decline in cognitive ability in the later [1]. This disease is first described by Alois Alzheimer in 1906, and two main pathological processes known as the deposition of tau and amyloid beta protein have been discovered during this over a century's related research [1]. However, though the understanding of AD pathogenesis stages further and many hypotheses have been put forward, there are still no efficient drugs that can alter the course of the disease. There are two current classes of drugs only, which are memantine and cholinesterase inhibitors and they simply act to control the symptom.

This review will overview the AD pathogenesis, summarize current pharmacological treatment, and highlight potential future therapies based on main mechanisms.

2. Pathogenesis

2.1. Mechanisms of apoptosis and pyroptosis

Apoptosis is a type of programmed death that exists throughout the life of multicellular organisms and can timely remove superfluous or damaged cells from the body. Currently known pathways that can

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DOI: 10.54254/2753-8818/21/20230865

mediate apoptosis include: the death receptor activation pathway, mitochondrial damage pathway, B granzyme signalling pathway, endoplasmic reticulum stress pathway and so on. Apoptosis is a pathophysiological stimulatory signal of the cell to the environment and is also a death process. In this process, cell contraction, chromatin condensation, nuclear segmentation, and finally cell decomposition into discrete membrane-bound apoptotic bodies will occur [2]. The induction of extracellular stimuli is crucial for cell apotosis.

2.1.1. Mitochondrial damage pathway. Apoptosis is initiated by the activation of the mitochondrial apoptotic pathway in response to internal apoptotic stimuli. This process is regulated by Bcl-2 proteins, and permeabilization of the outer mitochondrial motif leads to the incorporation of cytochrome c into the cytoplasm. A binding site for the adaptor protein Apaf-1 is present on the cytochrome c molecule, which, in response to ATP, combines with the 7-adaptor protein Apaf-1 in the cytosol, allowing it to undergo allosteric effects and become activated. Activated Apaf-1 aggregates and activates caspase-9 in a crad-crad manner to form apoptotic bodies composed of cytochrome c and others. Caspase-7, which is subject to this cleavage and becomes activated, causes downstream protein degradation related to cell life, ultimately causing apoptosis. Or apoptosis can also be mediated through the AIF protein. AIF can be released from mitochondria to the cytosol when cells are subjected to internal apoptotic stimuli and enters the nucleus and damages DNA, leading to cell death. In this process, bel-2-mediated MOMP is involved in the translocation of AIF from the inner mitochondrial to the cytosol [3].

2.1.2. Death receptor activation pathways. TNF receptors undergo oligomerization and structural changes by binding to associated ligands, exposing a DD capable of binding to adaptor proteins. When activated, adaptor proteins trigger the activation of downstream caspases, leading to apoptosis. Among them, Fas is the most studied death ligand. Its activation occurs by trimerization upon binding to the ligand FasL, and activated Fas can bind to and aggregate the Fas-associated death domain FADD through its DD, resulting in a change in the conformation of FADD. Whereas this change can lead to the activation of caspase-8, forming a protein complex (similar to the apoptosome) consisting of FasL, Fas, FADD, and Caspase-8, which can activate Caspase-3, triggering the cascade of apoptosis [3].

2.1.3. B Granzyme signaling pathway. Natural killer cells, like cytotoxic T cells, and lymphokine-activated killer cells, can induce apoptosis of target cells. FasL expressed on the surface of these cells binds FAS, and activates apoptotic pathways outside the target cells. Such toxic lymphocytes also deliver toxic particles to target cells, such as TNF, Granzyme B, and others. Perforin forms intermembrane channels on the target cell surface, facilitating the transfer of B granzyme to the interior of the target cell. Granzyme B has a proteolytic effect, it will cleave and activate caspases, and proceed apoptosis. Granzyme B mediates apoptotic effects more rapidly than direct activation of caspases by BH3-only proteins [3].

2.2. Endoplasmic reticulum stress pathway

The response of cells to endoplasmic reticulum stress is protective in nature. To prevent the accumulation of unfolded protein, the cells lower their concentration and inhibit the clumping of these proteins. When the endoplasmic reticulum becomes overloaded with misfolded proteins and there is a disturbance in calcium homeostasis, cells initiate various signaling pathways like unfolded protein response, endoplasmic reticulum overload reaction, and caspase-12 mediated apoptosis pathway as a reaction process. The ER stress induces the expression of glucose regulatory proteins GRP78 and GRP94 as endoplasmic reticulum molecular partners, leading to protective effects. However, it can also independently trigger cell apoptosis, impacting how the stress cells adapt, get injured, or undergo apoptosis in the long run.

Pathologically, Alzheimer's disease (AD) is characterized by the accumulation of hyperphosphorylated tau proteins to form Neurofibrillary tangles (NFTs) and the overproduction, oligopolization and deposition of β-amyloid (Aβ) of outside cells in the brain, resulting in a gradual

DOI: 10.54254/2753-8818/21/20230865

decline in cognitive function and the eventual onset of dementia [4]. NFTs of hyperphosphorylated tau proteins and amyloid plaques of insoluble Aβ peptides are seen on histopathological examination in AD. The accumulation of these abnormal proteins in the brain leads to neuroinflammation, glial cell activation and ultimately neurodegeneration. AD is associated with various disturbances in the molecular mechanism, resulting in ER stress. One such disturbance involves the aggregation of peptides, which can obstruct NMDA-R and trigger the entry of calcium into the cytoplasm. Disturbances in the amounts of the inositol-1,4,5-trisphosphate receptor (IP3-R) and ryanodine receptor (RyR) channels can cause disruptions in proper calcium regulation which can ultimately result in the demise of cells via the mitochondrial apoptotic pathway. The aggregate impact of these various cellular occurrences gives rise to long-standing ER stress [5]. The gradual accumulation and aggregation of hyperphosphorylated tau proteins or A peptides in AD cause irreversible ER stress, leading to synaptic dysfunction and ultimately, neurodegeneration.

The onset of AD is linked to the buildup of incorrectly shaped proteins in affected neurons, abnormalities in metabolism, heightened levels of oxidative stress, and a type of neuroinflammation that involves the glial cells in close proximity to these damaged neurons, including microglia and astrocytes. In this case, homeostasis is inhibited in the emergency room [4]. Thus, the AD brain exhibits ER stress.

2.3. The correlation between cell apoptosis and AD

Nowadays apoptotic mechanisms have been clearly demonstrated to play a major role in the pathogenesis of AD. Its process includes β- Amyloid aggregates and triggers apoptosis by inducing cellular early immediate response genes and their expression (c-fos, c-myc) by oxygen radicals. Oxidative stress has a great degree of correlation with it, and also studies have shown that oxidative free radicals are able to directly trigger the apoptotic mechanism and cause neuronal apoptosis [6].

2.4. Mechanisms of autophagy lysosomal pathway and ubiquitination proteasome system

Autophagy lysosomal pathway (ALP) and ubiquitination proteasome system (UPS) are related to AD or other neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), and prion disease.

Both ALP and UPS are crucial for the maintenance of cellular homeostasis by degrading and recycling cellular components to avoid waste. They both serve the same purpose: degrade the large protein complex into short peptides to be recycled and uptake by the cell for the synthesis of other proteins. They both experience the ubiquitination process, in which the substrate protein can be cascade marked with linear ubiquitin by three enzymes including E1, E2, and E3 as the target of degradation through the covalent combination with the ubiquitin molecule. As a result, the protein will be recognized by the 26S proteasome complex and be degraded.

In UPS, mono ubiquitin or polyubiquitin (Lys 11, 48) induces the target in the E3 stage. It uses proteasomes to degrade protein molecules. The proteasome is a combination of alpha and beta subunits in the beginning. It can switch to a 26S style with the help of ATP. The newly added 19S regulatory subunit can be functional in ubiquitin removal, the reorganization of substances, and the facilitation of protein unfolding. The unfolded protein will then be attached to the proteolytic site for degradation and the protein will be degraded into smaller peptides there. This process is also related to the cell cycle. It functions in degrading proteins responsible for pushing the cell into the next checkpoint. It also plays a role in antigen presentation. Intracellular proteins can be degraded into smaller peptides and presented onto the surface of the cell through this pathway.

The aggregation of insoluble protein in the brain nerve cells is a common symptom in AD and several other chronic neurodegenerative diseases. This aggregation could be due to the dysfunction or overburden of the UPS, or from structural or functional changes in the protein [7]. These two kinds of causes can disturb the normal recognition and degradation of protein by the UPS. The UPS takes part in maintaining the normal functions of synapses, so it is common that synaptic dysfunction is observed in AD since the deposition of insoluble protein aggregates in the brain of AD patients could defective proteolysis.

DOI: 10.54254/2753-8818/21/20230865

In ALP, polyubiquitin (Lys 48, 63) induces the target into ALP in the E3 stage. This autophagy-lysosome pathway is often responsible for those that cannot be easily targeted to the proteasome, such as larger protein aggregates, large organelles such as mitochondria, or more complex structures which may inhibit the association of proteasome onto these components. ALP often involves in the formation of a double membrane vesicle known as an autophagosome. It forms around the cellular component that needs to be degraded. Lysosomes then degraded the contents in autophagosomes into amino acids and fatty acids which can be recycled by the cell. This process is regulated tightly by complex protein networks and signal pathways including the AMPK pathway. It contains three different types, macroautophagy, chaperone-mediated autophagy, and microautophagy.

ALP involves in the degradation of proteins. The deficits in the ALP will lead to the aggregate of proteins, the generation of toxic protein species, and the accumulation of dysfunctional organelles, which are commonly witnessed in AD, PD, HD, and prion disease [8].

3. Treatment

3.1. Current treatments

Presently, there is no cure for AD and there are only some symptomatic treatments, most of which are moderate. The current treatment can be divided into two types: drug therapy and psychotherapy.

There are five drugs commonly used to improve cognitive impairment in AD, four of which are acetylcholinesterase inhibitors, and the other is an NMDA receptor antagonist.

In AD, the activity of choline neurons is reduced, resulting in a decline in acetylcholine concentration between synapses. To counteract this, acetylcholine esterase inhibitors can slow down the degradation rate of acetylcholine, which increases its concentration [9]. The first generation of cholinesterase inhibitors, Tacrine, had limited use due to hepatotoxicity [10]. Later on, Donepezil, Rivastigmine, and Galantamine were introduced and found to be effective in controlling the symptoms of mild and moderate AD, and there is evidence that they work for severe patients as well. However, these drugs do not delay the occurrence of AD in patients with mild intellectual disabilities. An excessive amount of choline can cause nausea and vomiting, which are the most frequently encountered side effects of these medications. Muscle cramps, bradycardia, reduced appetite and weight, and heightened production of gastric acid are among the less frequent side effects [11]. About 10% to 20% of patients may experience side effects that range from mild to moderate severity.

By non-competitively inhibiting NMDA receptors, memantine can prevent the loss of neurons and facilitate the functional recovery of damaged neurons, thus providing neuroprotection and improving symptoms. Its effectiveness has been demonstrated in moderate to severe AD, while its efficacy in mild AD remains unsupported. Moreover, the addition of memantine to polyperzane monotherapy may benefit individuals with mid-stage AD or cognitive impairment [9].

However, the efficacy of these drugs is not great, and so far, there is no evidence that these drugs can delay or stop the source of the disease.

Alternative therapies chosen by patients may include the use of the nutraceutical huperzine A, which is believed to improve memory function and daily activities. However, it is important to consider that its potency and purity may vary. It has been identified that a deficiency in vitamin D may increase the risk of developing dementia, and thus, patients who have been diagnosed with a deficiency are recommended to supplement an appropriate amount of vitamin D [12]. Adjuvant drug therapy, such as social psychotherapy, can be used to complement traditional drug therapy [11]. Social psychotherapy involves various interventional methods, including behavioural, emotional, cognitive, and stimulating therapies. Behavioural therapy aims to reduce specific symptoms by identifying the cause and effect of problematic behaviour. Emotional-oriented therapy includes several methods, but there is no conclusive evidence supporting their effectiveness. Cognitive-oriented therapy involves re-training patients to improve their mental functioning and restore reality orientation. Stimulation-oriented treatments, such as those involving art, music, pets, sports, or other recreational activities, may improve a patient's

DOI: 10.54254/2753-8818/21/20230865

behaviour, mood, and functions, although the efficacy of non-invasive and invasive brain stimulation on AD remains unclear.

Compared with people who do not exercise regularly, less atrophy is observed in the brains of patients with AD genetic risk factors, indicating that aerobic activity can prevent neurodegeneration [13]. Regular aerobic exercise can prevent metabolic diseases such as diabetes and also shows the maintenance of the body function of AD patients. As patients age, physical exercise can not only prevent the reduction or loss of strength and agility but also reduce neuropsychiatric symptoms and the care requirements associated with these problems [12].

3.2. Future treatment directions for AD

There is still great room for improvement in the medical direction of treating AD. The following are several currently popular and promising future methods for treating AD.

3.2.1. Therapeutic approaches related to traditional Chinese medicine for AD. With the improvement of the global medical level, the ageing of the population has gradually intensified, and the incidence rate of AD has increased significantly. At present, the global medical level has not reached the level of developing specific drugs to treat AD, and can only delay the course of the disease at most. One of the new treatment methods provides a good idea for the future treatment of AD, which is traditional Chinese medicine treatment. Traditional Chinese medicine has the advantages of stable therapeutic effects, low toxicity, multiple targets, multiple pathways, and multiple systems in improving AD symptoms [14].

From the aspect of TCM Pathology, AD is dominated by the loss of seminal oligo myelination and deficiency of Qi and blood, so there are methods for toning the spleen and stomach and kidneys. The spleen and kidney as the source of breath, blood biochemistry, and fine encapsulation, play an important role in solid fundamental, machine regulation. In traditional Chinese medicine (TCM), the treatment of ad is based on the spleen stomach and kidneys, and the most common formulas used clinically are qifuyin and Huanshaodan. Panax ginseng, Atractylodes macrocephala, and Glycyrrhiza consumed by Qi Fu are beneficial for robust spleen and stomach, staghorn gum, balanc gum, and acanthose gum can add refined Qi, and cooked Rehmannia is used for tonic intestine. Poria cocos, Z. jujuba, Chinese yam, Lycium barbarum etc. in also shaodan can gentle the body balance [14]. In response to this therapy, a well-known Chinese patent medicine, named ginseng Yang Rong decoction, was screened for experiments with mice as research samples. It was found that the decoction effectively improved spatial learning and memory ability, attenuated oxidative stress and hippocampal tissue damage in mice, increased ACh level in brain tissue, reduced AChE activity, and upregulated the protein expression of PSD95 and NR2B [15].

3.2.2. Insulin regulation is associated with future AD treatment. It has been observed that diabetes has the function of inducing AD pathogenesis. Mellitus generally results from inadequate insulin secretion. Its implication in AD is seen with insulin acting as a neuromodulator in the brain. Insulin dysregulation may contribute to AD pathology through several mechanisms, including reduced cortical glucose utilization. In addition to this, advanced glycation end products will increase oxidative stress, tau phosphorylation, and neurofibrillary tangles; While inhibition of insulin-degrading enzymes- increases β- Amyloid aggregation. Insulin and glucose regulation may emerge as future AD treatment options [16].

3.2.3. ALP. A major regulator of ALP is transcription factor EB (TFEB). This is a key factor in coordinating autophagy by regulating the formation of autophagosome and the fusion of autophagosome-lysosome positively through a selective combination with a promoter element called CLEAR [17, 18]. It also enhances cellular clearance through lysosomal exocytosis. It regulates metabolism and cellular clearance through lysosomal adaptation.

It has been verified that in multiple mouse models of AD for both Aβ and tau pathology, there is a beneficial effect mediated by TFEB.

DOI: 10.54254/2753-8818/21/20230865

For tau, through a TFEB–PTEN–Akt–mTOR–TFEB feedback regulatory loop, TFEB is further activated by the upregulation of PTEN and inhibition of Akt and mTOR induced by TFEB. As a result, the exogenous TFEB expression can enhance the ALP, and reduce tau pathology. For amyloid pathology, Aβ plaque pathology in the APP/PS1 mouse model can be reduced by a mechanism of astrocyte uptake and lysosomal degradation of Aβ, through TFEB expression in astrocytes [17].

There are many current treatments including Celastrol and a novel curcumin analogue C1, which are two kinds of TFEB agonists, and three-needle Electroacupuncture ameliorates (TNEA), which can be used to activate TFEB [18-20].

In summary, drugs that focus on TFEB might have a positive effect on AD and many treatments had put forward through this.

3.2.4. UPS. E3s mainly determine the specificity of substrates, so they are the best potential therapeutic targets among the enzymes that are responsible for combining ubiquitin with substrates.

Modulating the specific DUBs to enhance the deubiquitylation of polyubiquitin chains of mutant UBB+1, which inhibits proteasomes in the AD brain is another possibility.

Activation of the proteasome can also be an area of new drugs. It is a feature in AD patients that abnormal proteins aggregate or proteasomes inhibit. It could be efficient to remove the aggregated proteins or organelles that accumulate in the brain by enhancing proteasome activity in the following ways: by up-regulating the 19S and 20S complexes' assembly to increase the activity of the proteasome; by promoting the recognition of ubiquitylated proteins in protein aggregates; by modulating the chaperone or ATPase activity to unfold the aggregated proteins and by stimulating the catalytic activity of the proteasome [21].

In summary, drugs that focus on Ubiquitin-conjugating enzymes, DUBs and the proteasome might also have a positive effect on AD.

4. Conclusion

After collated studies of endoplasmic reticulum stress mechanisms, apoptosis and pyroptosis mechanisms, autophagy-lysosome pathway, and ubiquitination proteasome system mechanisms, all found their inseparable association with AD pathogenesis. This also drives further thoughts and actions on the treatment of AD. Existing pharmacological or psychological treatment modalities for ad including acetylcholinesterase inhibitors and NMDA receptor antagonists have nevertheless played a role in improving cognition in AD patients. The areas of TCM pharmacy, insulin mediation, ALP, and UPS that are mentioned will provide new ideas for both AD treatment and drug development for the future. And all the above treatment modalities are closely related to the mechanisms that lead to the pathogenesis of AD and play a regulatory role. It is believed that in the future, people's research on AD and the research and development of drugs will be further.

5. Authors' contribution

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

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230867

Natural product target on HIF inhibition

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Abstract. Lack of oxygen limits the growth of healthy cells. Surprisingly, however, 90% of solid tumors exhibit hypoxia, and this property of hypoxia is directly linked to tumor proliferation, differentiation, angiogenesis, energy consumption, the emergence of treatment resistance in cancer, and a worse prognosis for patients. However, as a tumor grows and oxygen levels drop, a gene called hypoxia-inducible factor (HIF1) is activated. Through its function as a transcriptional regulator, HIF1 reduces or even completely shuts down oxygen consumption processes in mitochondria, particularly oxidative phosphorylation, making glycolysis the primary source of energy for cancer cells. Given the link between cancer cells and HIF1, HIF1 is expected to be an effective pharmacological target for cancer therapy. This paper tends to introduce several inhibitors according to the characteristics of HIF1, which will provide a new idea for cancer treatment.

Keywords: HIF inhibition, natural product, HIF signaling pathway.

1. Introduction

Genetic mutations lead to unchecked growth and reproduction in human cells, which results in cancer. Human regular cells and malignant cells both require nutrition and oxygen to multiply. Once a cancer cell has been formed in the body, it divides and multiplies day and night while competing with healthy cells for nourishment. When the number of cancer cells grows large enough, tumor tissue can be seen. Tumor tissue must have an extremely high cell density to form, but because the body can only hold so much oxygen, the tumor tissue's microenvironment is anoxic. A key transcription factor that regulates cellular oxygen fluctuations and body homeostasis is HIF-1. It is composed of HIF-1a and HIF-1b subunits. Previous research has shown that the activity of HIF is controlled by the proteasome's response to the presence of oxygen in its component. The VHL E3 ubiquitin ligase interacts with HIFS at physiological oxygen levels, and the presence of ubiquitin causes HIF1a to become unstable and express less [1, 2].

By increasing the transcription of multiple oncogenic genes, HIF1 are essential for the adaptability of tumor cells to hypoxia and nutritional restriction [3]. Hypoxia is induced by tumor cells using a variety of ways. Through a variety of blood vessel-related effects, they disrupt oxygen transport or disrupt endothelial function, creating a chronic hypoxic environment, activating the hypoxia-inducing factor HIF signaling pathway, and increasing tumor development, invasiveness, and metastasis. As a result, HIF1 inhibition can be a useful target for cancer therapy. In order to achieve this, the various HIF1 synthesis and degradation processes will be examined below, and a summary of HIF-1 inhibitors in some natural products of the HIF1 signaling pathway will be given.

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DOI: 10.54254/2753-8818/21/20230867

2. Inhibitors of HIF-1α synthesis

The transcription, translation, and degradation processes for HIF-1 protein are the same as those for regular proteins. As a result, this section will discuss the HIF-1 protein's inhibitors in the transcription, translation, and modification processes.

The mechanism of HIF-1 protein synthesis that has drawn the most interest from researchers is the PI3K/AKT/mTOR signaling pathway, which regulates the translation of HIF-1A. Insulin, PC-3, DU145, EGF, and HIF-1 were all raised. These complexes increase the expression of erythropoietin, VEGF, and GLUT1, after they associate with the HRE and p300/CBP.

By inhibiting PI3K, the antibiotic wortmannin, which is produced by Penicillium wortmanni, prevents HIF1 nuclear accumulation. By inhibiting PI3K, rapamycin prevents insulin- and hypoxia-induced HIF-1 accumulation in human ARPE-19 cells and PC-3 cells. Everolimus (RAD001), an oral medicinal substitute for rapamycin, improved HIF-1 inhibitory effect in vivo and prevented the RMG-1 tumor from developing in ovarian clear cell adenocarcinoma.

Studies showed that PI3K/AKT/mTOR inhibition decreased HIF-1 activity, which in turn inhibits tumor angiogenesis. These compounds demonstrated hypoxia-selective growth inhibition and downregulation of the HIF-1. Further investigation indicated that the protein RPAP3, which results in mTOR dysfunction, is the precise target of dictyoceratins. It was discovered that verucopeptin, a cyclic isolated peptide from Actinomadura verrucosospora, inhibits the HIF-1 signaling pathway.

Verucopeptin suppresses HIF-1's transcriptional activity by inhibiting the accumulation. Six chiral centers were built to create the side chain unit of the verucopeptin, and the Fmoc-SPPS unit underwent macroscopic endocannabinoidization to produce the dipeptide core. The first comprehensive synthesis was finished in its last step by linking [4]. Verucopeptin, which has the ability to inhibit mTOR/p70S6K, has been demonstrated to have a considerable effect on the vesicular H+-ATPase subunit V1G in recent studies. V erucopeptin is resistant to human melanoma in vivo A375 and in vitro multidrug-resistant cancer cells. The plant-derived flavonoid apigenin and the plant antitoxin glyceollin I work together to inhibit the PI3K/AKT pathway and reduce the production of the HIF-1. Apigenin inhibits the angiogenesis of human ovarian cancer OVCAR-3, PC-3, and HUVEC tube-forming tumors in vivo. Both the mTORCI (mTOR/Raptor) and mTORC2 (mTOR/Rictor) complexes may include the mTOR kinase. The synthesis of the insulin receptor substrate-1 rises in response to the mTORCI inhibitor rapamycin, which prevents the feedback inhibition of this route and activates AKT. Rapamycin may, however, inhibit mTORC2 in particular cell types as the incubation time increases. Rapamycin inhibits VEGF synthesis during in vivo angiogenesis. mTOR is suppressed by the tsc2-TSC1 protein complex. VEGF and HIF-1 are highly produced by tsc2-deficient cells. Rapamycin treatment reduced VEGF levels but did not completely reduce HIF-1a levels. The mTOR signaling motif located at the N-terminal end of HIF-1 was showed to mediate the interaction between the mTOR regulatory-related protein (Raptor) and HIF-1. The production of VEGF is suppressed by rapamycin during in vivo angiogenesis. Simolimus, sometimes referred to as rapamycin, is a macrolide immunosuppressant that prolongs the lives of model species like mice by inhibiting the mTOR protein kinase [5]. The protein combination tsc2-TSC1 has a detrimental effect on mTOR. TSC2-deficient cells have large amounts of HIF-1 and VEGF. Rapamycin treatment reduced HIF-1a levels while also reducing VEGF levels in these cells. Raptor, a protein that controls mTOR, interacts with the mTOR signaling motif that may be present at the N-terminal end of HIF-1. In order for HIF-la to bind the coactivator CBP/p300 and work correctly in hypoxic conditions, this motif is required. The dual mTORC1/mTORC2 inhibitors OSI-027 and OXA-01 significantly reduce angiogenesis and regeneration, according to study [6].

3. Inhibitor of promoting HIF protein decomposition

Since HIF-1A is a protein, it is inevitably impacted by the processes of degradation. With increasing levels of degradation, HIFA expression declines. One of the most crucial elements in the stability of HIF-1 is the HSP90, a part of the cellular chaperone process. In addition to a number of other client proteins, HIF-1 has been discovered as an HSP90 client protein. The microbiological byproducts

DOI: 10.54254/2753-8818/21/20230867

geldanamycin and rhizomycin act as HSP90 inhibitors by tightly adhering to the ATP/ADP binding site. The HSP60 inhibitor epolactaene tert-butyl ester (ETB), which is made from the fungus metabolite epo-lactaene, supports the idea that HIF-1 protein downregulation mediated by HSP60 inhibitors. Though it was demonstrated that ETB inhibited chaperone proteins by binding to HSP60 Cys442, it's unknown how HSP60 and HIF-1 operate together [1].

TSA, a popular and typical histone deacetylase inhibitor, is a product of the fungus Streptomyces hygroscopicus. TSA was first found to increase the expression of VHL and decrease the levels of HIF-1 and VEGF in HepG2 cells. In a Lewis lung cancer mouse model, TSA also stopped the angiogenesis induced by hypoxia.

It has been discovered that ichthyone, antimycin A, and oligomycin are all naturally occurring inhibitors of mitochondrial complexes I, III, and V in Lonchocarpus utilis and other species. These mitochondrial electron transport chain inhibitors were found to impair the HIF-1 in a variety of cell lines. Saururus cernuus dineolignans manassantin B and its derivatives were shown to be strong inhibitors of HIF-1 that reduce mitochondrial oxygen consumption by bioassay-guided screening. By decreasing oxygen consumption or boosting reactive oxygen species, mitochondrial failure raises intracellular oxygen levels and destabilizes the HIF-1 protein.

4. Block downstream HIF-1 inhibitor

HIF-1 and HIF-1/ARNT form dimeric complexes with p300/CBP and engage in interaction before binding to HRE. Numerous organic substances have been identified as HIF-1 downstream inhibitors without affecting HIF-1 expression. Echinomycin is a naturally occurring, DNA-binding substance that was first discovered in Streptomyces echinococcosis. Echinomycin prevents DNA carrying the HRE consensus sequence from binding to HIF-1 in human glioma U251 cells, preventing HIF-1 from inducing the production of VEGF. It has been demonstrated that the fungus Trichoderma reesei's (chetomium sp.) metabolite chetomin interferes with the interaction between p300 and HIF-1. Additionally, Chetomin reduced HIF-1-mediated expression in both in vitro and in vivo tests, which prevented the development of HCT116 and PC-3 tumors from human colon cancer in vivo. By searching for HIF-1/HIF-1 dimerization inhibitors, the antibiotic/anticancer pigment acridine flavonoid was found. In addition to inhibiting spheroid formation (stem cell-like properties) in non-cancer stem cell (NCSC) and non-small cell lung cancer (NSCLC), chetomin, an active component of hair follicles, also reduces proliferation and chemoresistance in NCSC cultures of NSCLC [7].

Phosphoflavones directly bind to the HIF-1 PAS structural domain in vivo, limiting the downstream HIF-1 signaling cascade and preventing the development of the PC-3 tumor and the mobilization of angiogenic cells. The primary component of turmeric, curcumin, has several biological properties that have been well shown, including HIF-1 inhibitory action. In vivo, curcumin prevents the development of Hep3B tumors and HUVEC tubes brought on by hypoxia. The down-regulation of ARNT expression is one of the potential effects of curcumin [1]. The foundation of curcumin analogs is, however, somewhat shaky, and isolated curcumin (nearly invariably a combination of curcumin) has limitations as a workable therapeutic strategy [8].

5. Current application of HIF inhibitor in the field of cancer

In Hepatoma cells (HCC cells), the induction of HIF-1 and HIF-2 protein degradation inhibits the transcriptional activity of HIF. Inhibition of HIF-1 and HIF-2 activity in HCC tumors affects not only tumor growth and angiogenesis but also the immunological milieu of the tumor, enhancing antitumor immunity and enhancing response to anti-PD1 treatment. When CD47, CD73, and PDL1 are expressed in an HIF-dependent way, the capacity of the innate and adaptive immune systems of human breast cancer cells to eliminate cancer cells is diminished. This study demonstrates that BIRC2 expression in melanoma and breast cancer decreases CXCL9 expression and prevents the migration of NK cells and CD8+ T cells into tumors. In contrast, hypoxia did not cause the induction of BIRC2, CD47, or CD73 in cultured Hepa1-6 cells. However, 32-134D reduced the expression of the checkpoint ligands B7H4 and PDL1 as well as the checkpoint receptor TIM3 in Hepa1-6 tumors, both

DOI: 10.54254/2753-8818/21/20230867

of which were linked to HCC mortality. After 32-134D therapy, there was decreased production of the Th2 cytokines IL-4 and IL-13, which cooperate with CXCL1 to support or draw immunosuppressive MDSCs and TAMs. Treatment with 32-134D also resulted in a reduction in CD70 expression, which in glioblastoma and RCC contributes to immune evasion of cancer cells by triggering T cell failure or death. Cancer cells decrease immune function by competing with immune cells for the absorption of glucose (through SLC2A1/GLUT1), producing lactate (by LDHA), and creating an acidic extracellular environment (via CA9). The enhanced CD8+ T cell and NK cell recruitment caused by the increased CXCL9 and CXCL10 production in the tumors of 134D-treated mice accelerated the response to anti-PD1 therapy. Together, these studies have shown various immunosuppressive processes that are brought on by hypoxia in HCC and prevented by 32-134D therapy. Therefore, systemic HIF inhibition in HCC has the overall effect of reducing immunosuppression by promoting the tumor's recruitment of CD8+T and NK cells [9]. Numerous researches have looked at the function of HIF-1 in diverse metabolic reprogramming pathways during the past 20 years. HIF-1 restricts oxidative mitochondrial metabolism, promotes oxygen homeostasis under hypoxia, and inhibits oxidative mitochondrial metabolism by lowering oxygen consumption. This interaction between HIF-1 and mitochondria is critical for tumor cells facing hypoxia.

Significant advancements have been achieved in understanding of HIF-1, a crucial regulator of cancer development and a potential target for cancer treatment, in cancer cells. But there are some things about HIF membership that require clarification. For instance, the precise functions played by each family member and the interactions between HIF-1 and other family members (HIF-2 and HIF-3) during hypoxia adaptation. It's crucial to comprehend regulatory mechanisms in order to pinpoint precise treatment targets. Targeting hypoxia, which is closely related to HIF, is a potential therapeutic strategy to stop the spread of different malignancies and increase patient survival over the long term [10].

6. Conclusion

The intricate metabolic reprogramming that takes place in cancer cells that are repeatedly exposed to hypoxia has been emphasized in numerous studies. Through the oxygen-sensing PHD enzyme, the transcriptional complex hif serves as a key oxygen level sensor. Through mediation, HIF controls a variety of biological processes, such as cell division and metabolism. In this review, the focus was on the HIF-1 system's function in the metabolic adaptation of cancer cells to hypoxia, a process that is essential for promoting cancer cell survival, proliferation, and metastasis. Critical functions in the creation and suppression of cancer cells are played by hIF1 signaling pathways and downstream expression. Inhibiting PI3K/AKT/mTOR slows down HIF-1 activity, which in turn slows angiogenesis and tumor progression in terms of protein synthesis. Systemic HIF inhibition in HCC has the overall impact of reducing immunosuppression by promoting the tumor's recruitment of CD8+ T lymphocytes and NK cells. Fisetinone, antimycin A, TSA, and oligomycin each have a facilitative effect on HIF1 degradation and related organismal activity, respectively. Further testing of HIF signaling inhibitors may result in the discovery of novel potent HIF-targeted anticancer medicines with improved selectivity and reduced toxicity because natural compounds like microbial metabolites and plant metabolites have distinctive structures and intriguing modes of action.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230868

Applications of the CRISPR-Cas9 system in the treatment of KRAS mutated lung cancer

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Abstract. Lung cancer is currently the most prevailed cancer in men, and the second most prevailed cancer in women. It is by far the most fatal cancer with around 21% of overall death from cancer. It is very important to seek a way to treat lung cancers, since too many people died for lung cancers, too many families broke up and collapsed, and too many people are left with broken spirituality and heart. On the other hand, the successful treatment of lung cancer using method of gene editing could symbolize for the success of the appliance of gene editing on other deadly cancers. The main type of gene editing method that will be utilized is CRISPR-Cas9. This is a relatively new type of gene editing technology, thus it meant that this is the most applicable when coming to cost and precision, whereas it could also mean that it is still in its preform and hasn't been widely performed and tested. This review offers a brief introduction on lung cancer, summarizes CRISPR-Cas9's mechanism and provides the comparison of CRISPR-Cas9 on other gene editing technologies such as ZFNs and TALENs, sets up a plan to detect KRAS mutated lung cancer and apply CRISPR-Cas9 to treat KRAS mutated lung cancer in human cell lines and in chimpanzees.

Keywords: CRISPR-Cas9 system, lung cancer, small cell lung cancer, non-small cell lung cancer, KRAS mutation.

1. Introduction

As it is known, somatic cells go through cell cycle to divide using mitosis and cytokinesis. If under normal conditions without mutations, it divides in normal rate, then it is categorized as a healthy cell, however, if any part of the gene inside a normal cell mutates and cause the cell to undergo infinite divisions, then the mutated cell will be categorized as cancerous cell. For lung cancers, it often starts in bronchus, bronchiole, or alveoli [1, 2]. Lung cancers could also form when other part of one's body also has cancer, and the cancer cells travel through blood to reach the lungs [2]. Among lung cancers, there are two main types: The small cell lung cancer (SCLC), and the Non-small cell lung cancer (NSCLC). Among each of these, there are several subtypes. For NSCLC, it is the most prevalent type of cancer, adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, and sarcomatous carcinoma are the four sub types of NSCLC. For SCLC, it is harder to treat when compared to NSCLC, because it spreads and grow relatively quick, and small cell carcinoma made up this category [2]. There are also some other relatively uncommon types of lung cancers for example lymphomas, sarcomas, and pleural mesothelioma. For the five year survival rates of lung cancers, it is divided according to which stage the cancer is in: For one positioned (only in lungs), the 5 year survival rate is 61.2% overall (64% for

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DOI: 10.54254/2753-8818/21/20230868

NSCLC, and 29% for SCLC); For regional (spread to lymph nodes) lung cancers, the 5 year survival rate is 33.5% (37% for NSCLC, 18% for SCLC); For distant (cancer that spread to other parts of the body), the 5 year survival rate is 7% (26% for NSCLC, and 3% for SCLC) [2]. There are a lot of risk factors of different types of lung cancers, with these being the most common ones: Smoking tobacco products, inhaling second-hand tobacco smokes, exposed in bad environments for example air pollution, radiation on lungs, and family inheritance of lung cancer genes. The rate that people develop lung cancer is surprisingly high: almost 1 in 16 people will be diagnosed with lung cancer in their lifetime. Common symptoms could be shown when one is diagnosed with lung cancer are Blood when one cough or spit, respiratory infections, and inflammations frequently, lasting coughs, pains in chests, backs, and shoulders, breathing troubles, hoarse voice when speaking or breathing, and appetite loss, weight loss, exhaustion [3]. Some common ways to diagnose lung cancers are Blood test that can tell one how his/her organ are working; Imaging which can provide clear scanned image of one's lungs; Different types of biopsies that can help doctors check what type of lung cancer is developing; And also the molecular tests that can tell which gene is mutated that caused lung cancer [3].

2. Basic information of CRISPR-Cas9 system

2.1. CRISPR/Cas9 system

Clustered Regularly Interspaces Short Palindromic Repeats (CRISPR) is originally adaptive immunity in prokaryotic cells, discovered by Atsuo Nakata group in Osaka University. It functions as follows: When a strand of DNA is extracted, it will be shown as: unique gene sequences that are foreign will be nestled between each palindromic repeated gene sequence named spacers. In these kinds of bacteria, once the bacteriophage attaches to the surface of the bacteria, it releases viral DNA into the bacteria to try to kill the bacteria. However, after the viral DNA is released, it is then inserted between the palindromic repeats to become a spacer which is called the CRISPR array. Then, due to central dogma, this CRISPR array will then be transcribed into RNA, which is called the pre-crRNA, or simply, crRNA ("cr" here stands for CRISPR). Then the Cas9 enzyme gets involved (Cas9 stands for CRISPR associated nuclease), also together with it, there is a tracrRNA.

This RNA can bind to the palindromic repeats. And thus, there is a complex consisting of one sequence of pre-crRNA (made with one spacer and one palindromic repeat), one Cas9 and one tracrRNA. There is then the Ribonuclease three (RNase III), to cut the rest of the gene sequences between each effector complexes. Each effector complexes provide protection from foreign invasions in the following ways: If the viral DNA from the outside gets in contact with those effector complex, and the gene sequence is complementary to the gene sequence in those effector complex, then those sequence will combine. After it combined, the cutter inside the Cas9 complex will cleave the sequence for around 20 base pairs long, and by few base pairs upstream of the protospacer adjacent motif (PAM) sequence.

Thus, a double stranded break is inducted. After the double stranded break is inducted, the double stranded gene can then repair through one in these two repair mechanisms: Non-Homology Directed Repair (NHEJ), or Homoly Directed Repair (HDR). NHEJ is done by directly ligating each of the end of the double stranded break without guidance [4], and some of NHEJ can introduce insertion or deletion of nucleotide, which is referred to as: indels. Thus, NHEJ produces repaired strands in non-uniform lengths. On the other hand, HDR is a lot more precise way to repair double stranded break by using either an endogenous DNA template or exogenous DNA template [5]. Since HDR doesn't require indels, the length of repaired gene sequence is uniform. For these two repair mechanisms, it happens randomly inside the gene sequences after editing. The NHEJ method is more error prone, while the HDR is less error prone.

2.2. Advantages of CRISPR/Cas9 system

After the proposal proposed by Jennifer Doudna and Emmanuelle Charpentier on CRISPR-Cas9 system could potentially edit genes of human, scientists began actively investigating in the CRISPR-Cas9 system. Soon, the scientists found out that tracrRNA and crRNA could be combined, thus lead to better

DOI: 10.54254/2753-8818/21/20230868

coordination. Using a linker, tracrRNA and crRNA could be combined into a single guide RNA (sgRNA). This sgRNA has the same function as both the tracrRNA and crRNA have. This is a major breakthrough in the field because this means that any gene sequence of about 20 base pairs could be targeted just by simply synthesizing the corresponding sgRNAs that complements the gene sequence that needs to be targeted.

Zinc finger nucleases (ZFN), transcriptional activators like effector nucleases (TALEN), and CRISPR are examples of artificial nucleases that have revolutionized biological research. In actuality, the capacity for disease study and treatment has been substantially increased by these cutting-edge technologies. For ZFNs, it works by binding zinc finger protein to a Fok1 enzyme to target a specific gene sequence. TALENs works by Cooperating a transcription activator like effector with a nuclease enzyme to recognize a mutated gene sequence and create a double stranded break to it. Eaches' limitation is obvious: For TALENs, it targets only one site at one time, so it is relatively low in efficiency [6]. Also, scientists need to design a long RNA sequence by designing every single base pair, thus, it is really time consuming and cost a lot too [6]. ZFN has the same problem as TALENs. In comparison, CRISPR-Cas9 complex is a lot easier to use and a lot cheaper. This is because CRISPR only has to design the corresponding single guide RNAs for a random of 20 base pairs that the Cas9 complex wants to target. However, TALENs and ZFNs needs to design base pairs one by one in order for precise targeting [6].

3. Applications of CRISPR/Cas9 system in treating lung cancer

KRAS is an enzyme in a form of GTPase, and this serves as sending information from outside into the cell. When it mutated, it will signal too much to make the cell divide too much, thus, will cause the cell to divide as cancer tumors. There are already a lot of treatments regarding to this problem, for example: Chemotherapy, radiation therapy, and surgical therapy. Those all have limitations when compared to gene editing therapy: Chemotherapy is mainly on taking pills and injecting medicines in a try to kill cancer cells. However, the cancer cells grow at a lot faster rate that the rate the medicine kills the cells, and, some cancer cells can grow immune to the medicine. Plus, when chemotherapy is used in long term, it may cause a lot of stress to one's body, leading problems to stomach, skin, nail, etc [7]. Second, radiation therapy also has a lot of setbacks, for example, while killing the cancer cells using high energy radiation, it also kills healthy cells near the cancer cells. Thus, a big burden will be laid off on the patients. Last but not least, is the surgical therapy, this also has drawbacks because doctor may not cut all the cancerous tumors, and also, patients may have a big chance of dying during the surgery, because cancers are related with blood vessels, thus cutting may results in hemorrhoea. There are four main steps in detecting and treating the KRAS mutated lung cancer: Detection using NGS, safety test, CRISPR Knock Out, and finally take in pills.

3.1. Detection using NGS

First of all, the detection of the KRAS mutation can be done by using comprehensive next generation sequencing (NGS: a piece of technology that helps to determine the sequence of DNA or RNA to study for certain diseases) [8]. NGS requires mainly 4 steps: The extraction of DNA or RNA pieces from nucleic acids, which means we have to take nucleic acids from patients and extracts its DNA; Then, library preparation is needed and could be done by cutting the KRAS mutated samples of DNA extracted from step one into small fragments and adding one adapter at both ends of the fragment which could be used later for the NGS to easily detect; The third step is to amplify the KRAS mutated fragments that needed to be tested by attaching them to beads that might help to increase the fluorescence of the KRAS mutated fragments, and make the machine easier to detect; Last but not least, processing, analysis, and interpretation are done to fully to the results shown to ensure that the patients do have a KRAS mutation. The NGS also has a clear advantage when compared to some other sequencing methods for example: Sanger sequencing, microarrays, and PCRs, where NGS can provide sequencing to tons of DNA fragments, while at the same time, it ensures the speed and accuracy of the sequencing, also minimizing the cost.

DOI: 10.54254/2753-8818/21/20230868

3.2. Safety test

Secondly, before cutting the DNA using Cas9 nuclease, several things needed to be checked to ensure that the editing is safe: First, we need to test for the specificity of the editing. The way that it works is as follows: Create a cell line that contains normal healthy cells, and KRAS mutated cells. Insert the Cas9 complex together with the sgRNA inside to both of the cell line using different transfection methods [9]. If the Cas9 nuclease only do editing on the KRAS mutated cells, then it is proven to be useful; At last, the designed guide RNAs needed to be tested on to prove whether it is useful for treating the cancer and letting the tumor become smaller, or they are not useful, and the tumor kept growing bigger and bigger.

3.3. CRISPR Knock Out

Third, which is the most important step, is the utilization of CRISPR Knock Out (CRISPRko) to correct the KRAS mutated gene. This is done by using active Cas9 nuclease and the sgRNA that is specifically designed to complement the gene sequence of the cell extracted from patients' body. In this case, when the complex moves near to the KRAS mutated gene sequence, it detects it, and the sgRNA will unwind the double helix DNA strand. After that, the sgRNA will attach to the gene sequence beside the PAM sequence, and the cleaving domain one the Cas9 will corporate to cleave the DNA strand to make a double stranded break. After the double stranded break is induced on the gene sequence, the cancerous gene won't work anymore. And last, the broken double strand will repair either by non-homologous end joining, or by homology directed repair.

3.4. Take in pills

Finally, after the KRAS gene won't work anymore, the cancer cells stop dividing, pill intaking or injection would then be useful, because the cells stopped proliferating.

After the investigation is done on human cell lines, it is time to give it a shot on animals for example the chimpanzees. According to research, chimpanzee has 98% of the DNA same as humans' DNA [10]. We can search for chimpanzees that has been infected with KRAS mutated genes that leads to lung cancer, and the chimpanzees that are normal. Then, we need to extract the DNA strands in both type of the chimpanzees to create a cell line that is consisted of both KRAS mutated and normal cells. This is when we add the Cas9 complex with specifically built sgRNAs to the cell lines: If the normal cells' KRAS kept signaling at the same normal pace, while the KRAS mutated cells has the KRAS signaling frequency reduced to zero, it is said to be specific, and successful, and if it both end the KRAS signaling or has no effect on KRAS signaling in either cell, it is said to be unsuccessful and unspecific. In the second test, we are aiming for efficiency, which can be defined as how precisely the sgRNAs pair up with the sequences: First, clone the sgRNA into a container that has Cas9, and U6 promoter in, then, check the fluorescent cells, grow it, and at last, put it in test using PCR [7]. Once the test results shown that the sgRNAs designed are both specific and efficient on the chimpanzees, this could then be tested on human. This could possibly also apply to other animals too.

4. Conclusions

In conclusion, lung cancers, are divided into NSCLC, including the specific mutation in the KRAS gene mentioned in the whole passage and SCLC. CRISPR system, which is originally identified as immune system in prokaryotic cells, has the advantage of cheaper and easier to use compared with ZFNs and TALENs. The treatment of this gene-editing technology to lung cancers involves 4 steps: Using NGS to determine which part of the gene mutated, secondly, the designed sgRNAs needed to be tested on specificity of editing and effectiveness of the RNAs on editing mutated genes. The third step is to insert the Cas9 complex into the cancer cells and provide an environment that allows it to perform gene editing. And last but not least, after the gene essential for tumor growth has been cut, pills could be retaken and will be effective. After human cell line investigation proved to be applicable, it could also be applicable on animals that has similar genes as humans for example the chimpanzees. When people look at the potential future of using the CRISPR-Cas9 system to cure diseases, there are a lot of people still need to consider on: Designing sgRNAs precisely so that they can pair up with gene sequences effectively but

DOI: 10.54254/2753-8818/21/20230868

not off target, reducing the possibility of inducing unintended change in human embryos, and ethical issues etc. But after these all are solved, human will experience a big increase in overall wellbeing, health care level will drastically improve, average human life span will increase, and people no long have to fear for those fatal diseases out in the world. Thus, scientists should break more into this field, experience more, investigate more. Outside of contributing to medication technology, the applicability of CRISPR-Cas9 in gene editing could also potentially help scientists to use this technology to maintain ecosystem stability.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230871

Role of adipose-derived stem cells in osteoarthritis

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Abstract. Osteoarthritis (OA), a widespread degenerative condition affecting the joints, is especially prevalent in the elderly population. While pharmacological interventions are available to alleviate pain associated with this condition, their therapeutic efficacy remains limited. Furthermore, the potential for adverse reactions may inhibit satisfactory clinical outcomes in OA treatment. Recent studies indicate adipose-derived stem cells (ADSCs), due to their ease of procurement and expandability, as a promising alternative. These cells serve as an essential source for bone tissue engineering, given their ability to differentiate into various cell types, and thus, their transplantation may offer a novel approach in OA treatment. The relative abundance of ADSCs in human and animal bodies, coupled with the ease of material collection, lends them unique advantages. This review explores the pathogenesis of osteoarthritis, the biological characteristics of ADSCs, mechanisms of chondrogenesis, and their therapeutic potential for OA. We also delve into various culture methods associated with these cells, with the objective of offering fresh insights for clinical OA management.

Keywords: osteoarthritis, adipose-derived stem cells, chondro-differentiation.

1. Introduction

OA is a common degenerative condition that impairs the health of the bones and joints, affecting approximately 7% of the global population with differing degrees of severity. Those aged 65 and older are disproportionately affected. The pathogenesis of osteoarthritis is multifactorial, involving a complex interaction between factors including aging, genetics, trauma, and obesity. The disease is distinguished by pathological alterations in all joint tissues, including cartilage, subchondral bone, ligaments, meniscus, joint capsule, and synovium [1]. Osteoarthritis imposes a significant burden on patients and their families, necessitating the development of effective treatment methods. Although surgical interventions and medications are available, the need for improved therapeutic efficacy remains.

In this context, mesenchymal stem cells (MSCs) arise as a promising technique for cartilage repair due to their capacity to differentiate into bone and cartilage cells and to secrete substances that promote tissue repair. MSCs are increasingly recognized for their role in osteoarthritis treatment due to their regenerative, anti-apoptotic, anti-inflammatory, anti-aging capabilities, and paracrine functions. MSCs considerably contribute to the improvement of osteoarthritis patients' quality of life by alleviating symptoms and enhancing joint function.

ADSCs, which are pluripotent cells that originate from adipose tissue and can differentiate into multiple cell types, are of particular interest. ADSCs exhibit minimal immunogenicity, maintain similar self-renewal capacities, and can be derived from numerous tissues, including bone marrow, adipose

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DOI: 10.54254/2753-8818/21/20230871

tissue, umbilical cord blood, and placenta. They perform essential roles in vital biological processes, such as angiogenesis, cell division, differentiation, and inflammation control. Recent research indicates that chondrocytes derived from ADSCs can extend the lifespan of osteoarthritic chondrocytes by delaying the onset of aging markers caused by inflammatory stress, thereby potentially contributing to the therapeutic effect. In addition, it is believed that ADSCs release signals that promote the growth of adipose tissue and blood vessels, suggesting a function in healing processes. Notably, following ADSC transplants, the body exhibits decreased rejection rates.

Consequently, this review aims to assess the progress of research and development in the field of ADSC therapy for osteoarthritis, to explore the dependable therapeutic directions and mechanisms of osteoarthritis, and to highlight the significance of research in bone tissue engineering and regeneration pathogenesis of OA.

Osteoarthritis was originally perceived as a disease of "wear and tear". The primary drivers behind this condition were thought to be chronic loading and compromised joint biomechanics, leading to the breakdown of articular cartilage and subsequent inflammation. This process would then result in stiffness, swelling, and loss of mobility. However, contemporary understanding posits that the pathogenesis of osteoarthritis involves both inflammatory and metabolic components [2]. One of the most detrimental effects of osteoarthritis is the substantial degeneration of articular cartilage that occurs over the disease's duration. This cartilage serves a crucial function by facilitating low-friction movement within joints and evenly distributing load-bearing stress. When osteoarthritis impacts a joint, it affects not only the cartilage but also the synovium, articular ligaments, and subchondral bone [3]. Various theories attempt to explain the underlying mechanism of osteoarthritis. One such theory suggests that the disintegration of cartilage triggers a foreign body response in synovial cells. This reaction instigates the production of proteases, spurs synovial angiogenesis, and leads to the secretion of inflammatory cytokines, which in turn contribute to further cartilage degradation. Other hypotheses emphasize the significant role played by activated synovial macrophages and the innate immune system in the progression of the disease [1].

2. Biological characteristics ADSCs

Given the right inducing conditions in a lab environment, these cells have the capacity to morph into various other cell types such as osteoblasts, chondrocytes, adipocytes, muscle cells, and nerve cells. Moreover, they have the ability to excrete an array of factors that promote angiogenesis and inhibit apoptosis, potentially contributing to cell survival, tissue recovery, and regrowth. The attributes of adipose tissue, such as ease of acquisition, ample cell count, broad availability, low risk of immune response, and stable cell characteristics, have propelled it to become a focal point in tissue engineering stem cell research in recent times [4].

3. Mechanism of ADSCs chondrogenesis

As per the findings presented by Antonio et al. [5] ADSCs can serve a protective function for various innate tissues. They exhibit immunoregulatory capabilities and can aid in tissue regeneration via soluble elements. The secretory proteome of ASCs, as identified, contains a wide range of growth factors. Some examples of these include HGF, GM-CSF, IL 6, 7, 8, and 11, TNF-α, VEGF, BDNF, NGF, and adipokines. Under hypoxic conditions, when HIF-1-alpha stabilizes, there is an observed increase in the secretion of VEGF by ADSCs.

According to a study referenced [6], VEGF is a crucial factor in bone regeneration, contributing to osteogenesis, and it is also instrumental in the creation of new blood vessels via the process of angiogenesis. Hans-Peter's experiment of inactivation of VEGF in mice showed that VEGF was a crucial signal regulating growth plate morphogenesis and inducing cartilage remodeling, which proved the importance of VEGF in chondrocyte breaking function and extracellular matrix remodeling [7]. VEGF also exists in fully mature hypertrophic chondrocytes [8]. The extracellular matrix of rat adipogenic stem cells was removed by collagenic enzyme digestion and gradually purified, and the fourth-generation ADSCs were bone induced, resulting in cell calcification, which proved again that ADSCs

DOI: 10.54254/2753-8818/21/20230871

can perform bone differentiation and cartilage differentiation by inducing ASCs, and regenerate new cartilage tissue.

4. Mechanism of ADSCs in treating osteoarthritis

Recent research has demonstrated the paracrine function of ADSCs in preventing chondrocyte degeneration, while VEGF has also been proven to be a key regulator of ADSCs playings a paracrine role [9, 10]. With age, cartilage degrades and loses its matrix, and cartilage cells produce inflammatory mediators, leading to serious damage. In older adults, surgery should be avoided whenever possible, and some procedures only rebuild degraded cartilage, but do not slow or stop the process of joint inflammation that has built up. In the experiment of Hirotaka et al. [11], it was proved that the higher VEGF protein level in the ASCs transplantation group and the in vitro experimental results of ASCs conditioned medium supported the paracrine mechanism. In a study conducted by Li Mei et al. [12], an investigation into OA in rats was carried out. The findings indicated that, relative to the IL-1β +ADCS treatment group and the non-treated control group, TNF- and IL-6 mRNA expression levels both significantly decreased. However, no discernible rise in the expression of IL-10 was seen. From these observations, it can be tentatively inferred that ADSCs contribute to the deceleration of joint inflammation progression through paracrine mechanisms. Further investigation by Kuroda Isari et al. supports this view. The team injected ADSCs into the joints of albino rabbits. The ADSCs were found to home into the intra-articular soft tissues (including the subintima of the synovium and ligament) 8 weeks post anterior cruciate ligament injury (ACLI). The results suggested that the paracrine actions of ADSCs residing in the intra-articular soft tissues significantly aid in impeding the progression of cartilage degradation.

5. In vitro culture method of ADSCs

In the human body, there are usually three different types of fat cells: white, brown, and beige. The white fat, which is found in the arms, thighs, hips, and belly, is made up of white cells that are kept under the skin or close to the organs. Brown fat is a type of fat that helps the body store energy and keep it warm. It is usually found in babies, but also in adults to a lesser extent, mainly in the neck and shoulders. Beige fat functions halfway between brown fat cells and white fat cells. Beige cells can help burn fat instead of storing it. ADSCs are usually derived from white fat and are separated from the fat tissue located in the abdomen by procedures such as subcutaneous liposuction or direct excision techniques. After the adipose tissue is obtained, it is first washed with a phosphate buffered saline solution. The required stromal vascular fraction (SVF) was then separated by collagenase treatment, centrifugation, washing and filtration, refer to Figure 1 for detailed steps. From the research conducted by Lee et al. [13], they deduced that the optimal condition for the separation of the stromal vascular fraction (SVF) was a treatment duration of 40 minutes with 0.1% type I collagenase. Refer to the image below for further clarification. The SVF is comprised of various cell types, including ADCS, fibroblasts, monocytes, macrophages, lymphocytes, and preadipocytes. These SVFs were subsequently seeded in petri dishes and cultured under conditions of 37℃ and 5% CO2. The culture medium was replaced every three days. Upon reaching a cell growth of 80%-90%, passaging was performed, and the cells were subsequently treated with Gibco BRL for passaging culture [13, 14].

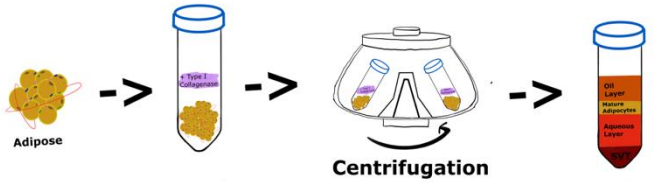

Figure1. Methods and procedures of ADSCs extraction.

DOI: 10.54254/2753-8818/21/20230871

6. Osteogenic induction and morphological changes of ADSCs

Cells from the fourth generation were procured and introduced into an osteogenic induction medium. This medium was composed of fetal bovine serum, dexamethasone, ascorbic acid, and β-sodium glycerophosphate. These components were utilized for the purpose of fostering osteogenic induction culture. 14,28 days after osteogenic induction, alkaline phosphatase and cicarin red were stained, respectively, and glycerin gelatin sealed. After 2 weeks of osteogenic induction, the cell morphology changed, showing irregular shape or polygonal shape, and the cell volume increased slightly. Four weeks after osteogenic induction, red mineralized nodules were observed by alizarin red staining, and the intracellular alkaline phosphatase activity was measured by calcium and cobalt staining, and graying black granular or massive precipitates were observed in the cytoplasm [15].

7. Conclusion

In patients with OA, the only treatment currently available cannot completely prevent the progression of joint degeneration. The treatment of cartilage defect and joint degeneration by ADSCs is a hot research point in the field of orthopedics. The benefits of ADSCs include facile sampling, little patient damage, good cell differentiation capacity, difficult apoptosis, and promotion of cartilage proliferation and differentiation. ADSCs can secrete a variety of cytokines during proliferation and differentiation, which can promote cartilage differentiation, chondroblast related gene expression, chondrocyte proliferation, and play a role in promoting cartilage regeneration and repair. At the same time, ADSCs also have paracrine effects on chondrocytes, which can not only regulate the proliferation of related cells and improve clinical symptoms, but also promote angiogenesis and tissue survival. Despite the fact that the mechanism through which ADSCs cure OA is not yet fully understood, its role in promoting cartilage differentiation and regeneration has been confirmed. Further progress in terminating OA or even reversing the development of OA has been made possible. However, there are still many problems to be solved, such as the optimal sampling site of ADSCs, the application mode of OA treatment, dosage and course of treatment, specific molecular markers of ADSCs, the molecular mechanism of ADSCs proliferation and differentiation, the safety assessment of ADSCs in vitro culture, and the selection of the optimal transplantation route and time window of ADCS. There is reason to believe that further research on the clinical transformation of ADSCs will promote the development of medical technology and open up a new way for the treatment of OA in the future.

Authors contribution

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

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230872

Brief summary on the mechanism, symptoms, epidemiology, management and treatment of osteoarthritis

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Abstract. The most common degenerative joint disorder is osteoarthritis (OA) which impacts hundreds of millions worldwide, with the primary risk factor being age. The disorder is characterized by the breakdown of extracellular matrix (ECM) components, leading to impaired chondrocyte function and cartilage repair. Further, the subsequent inflammation results in pain and even joint disability in severe cases. Conventional treatments focus on symptomatic relief and improvement of joint function, through pharmacological means, physical therapy or surgeries. However, long-term anti-inflammatory or analgesic treatment may result in adverse side effects such as drug dependence. Meanwhile, joint replacement surgery poses risks of permanent disability. This review discusses the underlying mechanisms and risk factors of OA, their relationship to symptoms and disease progression, and current epidemiological trends. Additionally, we focus on new-generation treatments with the application of bioengineering and stem cell therapy. Finally, we address contemporary treatment strategies and the potential for future disease-altering therapies that may be implemented on a large scale.

Keywords: osteoarthritis, bioengineer therapy, stem cell therapy.

1. Introduction

Osteoarthritis (OA) affects 600 million people worldwide as the most common joint disorder. It primarily affects articular cartilage, bones, and the surrounding soft tissues. The normal function of the articular cartilage of the joints is by the chondrocytes which produce and maintain the cartilage through its anabolisms and catabolism. In OA, there is a breakdown of extracellular matrix (ECM) components such as collagen and proteoglycans. This results in a decrease in ECM synthesis, therefore, a loss of chondrocyte function and capability for cartilage repair. During the response to stimulation, inflammatory cytokines are released in the joint, inducing inflammation, and further promoting disease progression. Moreover, the resulting loss of chondrocyte function has been linked to the risk factors of ageing, genetic factors and oxidative stress [1]. The main risk factor for OA is ageing, which is important given the ageing population of the world [2]. Conventional treatment relies on pharmacological therapies to provide symptom relief through the improvement of joint function, allowing patients to resume an active lifestyle, which is essential for maintaining joint stability. However, long-term use of pain medications, especially opioids and NSAIDs, may cause side effects or even drug dependence. As a last resort, arthroplasty (joint replacement surgery) may be considered, restoring a modicum of joint function, at the risk of permanent joint disability. New therapies currently under development would allow the regeneration of cartilage without the risk of major surgery. In this review, we summarize the

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DOI: 10.54254/2753-8818/21/20230872

mechanism and the symptoms of OA. Moreover, we focus on the disease progression and outcomes, the relationship of the current trends in epidemiology and rising importance. Importantly, we discuss the contemporary treatments and management of symptoms concerning possible disease-altering treatments that can be implemented in the future.

2. Epidemiology of osteoarthritis

OA can significantly impact the quality of life of affected individuals. The epidemiology of OA is essential given its significant and growing impact on public health worldwide. According to WHO, the World Health Organization, it was estimated that in 2020 one billion were aged 60 or older, comprising just under one-eighth of the global population; it is projected to increase to one-sixth by 2030. In 2019 with 528 million people with OA, approximately seven per cent of the world's population. As the risk for OA increases with age, it is a substantial contributing factor to disability in older adults.

Additionally, OA is more prevalent throughout developed countries, most likely due to longer life expectancies along with greater exposure to risk factors such as obesity and physical inactivity. OA is the most frequently occurring joint disorder in the United States, affecting about 30 million adults. In Europe, over the age of 60, OA impacts women more than men with 18% of women compared to 10% of men, which is expected to rise by 40% for both sexes in the next 20 years. However, OA is less common in Asia than in more industrialized countries, although it is also on the rise due to ageing populations and changes in lifestyle factors.

3. The mechanism and clinical features of osteoarthritis

The underlying mechanism of biomechanical pressures on the hyaline cartilage in joints influences the ECM's combination of chemical and biological factors. Since cartilage's mechanical properties are provided by ECM, this results in the resulting breakdown. Generally, proteoglycans are replaced at a faster rate than collagen proteins. When collagen degeneration increases, it is replaced with type one collagen, which is not as mechanically suited for the role and wears away faster [2]. As the rate of matrix breakdown exceeds the anabolic repair process, the cartilage gradually decreases in depth and eventually reaches the bone in late cases [1]. The secretion of inflammatory cytokines in response to joint injury or chronic mechanical stress intensifies the breakdown of cartilage and other joint tissues, further leading to pain and joint disability. The inflammation and oxidative stress that contribute to chondrocyte dysfunction and matrix breakdown involve the activation of matrix metalloproteinases (MMPs) and aggrecans [3]. These enzymes further degrade the ECM, leading to the release of fragments that can trigger more inflammation and catabolic processes, creating a vicious cycle of damage [2]. In advanced stages of OA, the subchondral bone can change such as sclerosis and cyst formation, further compromising joint integrity. Meanwhile, the formation of osteophytes, bone spurs, and synovitis (inflammation of the synovial membrane) contribute to pain and stiffness.

Generally, although symptoms may vary depending on the location, characteristic joint pain, stiffness and subsequent joint disability, especially in the morning or after a period of inactivity. It is noted to improve during physical activity. There may also be a feeling of grinding and popping in the joint known as crepitus. In the most advanced cases, as the cartilage wears down osteophytes (bone spurs) will appear, a sign of late-stage OA, restricting the range of motion through inflammation and resulting in external deformities of the fingers. Inflammation also occurs though less so than in rheumatoid arthritis resulting in swelling warmth and tenderness.

4. The risk factors of osteoarthritis

Common risk factors of OA include obesity and those who have suffered specific joint trauma in the past causing joint misalignment. The risk factors can be in two categories physiological and local (joint level), the strongest risk factor for OA is age, although obesity also plays a role in OA cases in a large proportion of weight-bearing joints such as the hips. Age shows a positive correlation with the degree of symptoms. Obesity is another significant risk factor for OA, particularly in weight-bearing joints such as the hips and knees, although there is mixed evidence for the hip joints. The excess body weight places

DOI: 10.54254/2753-8818/21/20230872

additional stress on the joints, increasing the likelihood of matrix breakdown. Genetics can also play a role in OA development, as certain genetic factors may predispose individuals to OA. Women have a higher chance of developing OA than men, particularly after menopause due to the decrease in hormones that maintain bone density. Hip, knee and hand OA are more commonly diagnosed in women than men. Other indirect factors could also contribute to the higher risk, for example, reduced cartilage volume, bone density and muscle mass in comparison. Acute joint trauma, such as a ligament tear or fracture is common in athletes, and can also increase the risk of developing OA in that joint. Chronic mechanical stress, such as kneeling or squatting, can also contribute to the development of OA in the affected joints.

5. The therapies of osteoarthritis

5.1. Pharmacological treatment and surgery

Treatment options for OA include lifestyle modifications, physical therapy, pain management, and joint replacement surgery in severe cases [1]. Pharmacological therapy is employed as the initial treatment for OA, with different classes of drugs employed depending on the patient's pre-existing conditions and the severity of their OA symptoms. Paracetamol is a typical over-the-counter painkiller used to treat mild to moderate OA pain. It has fewer adverse effects than other pain medications such as NSAIDs (Nonsteroidal anti-inflammatory drugs) or opioids but may be less effective in treating severe pain. Traditionally, NSAIDs, like ibuprofen and naproxen are used, as they are effective in relieving moderate to severe OA pain and inflammation, but they can have gastrointestinal adverse effects, especially when used long-term. While opioids such as tramadol and oxycodone may be administered for severe OA pain, their chronic use is controversial due to their potential for addiction and other negative effects [4]. To avoid such severe side effects, alternative treatments such as topical analgesics, are considered. The usage of these analgesics could treat OA pain with fewer adverse effects than systemic medications. The typical topical analgesics are capsaicin and lidocaine formulations applied directly to the injured joint to deliver regional pain relief with no systemic adverse effects. Intra-articular corticosteroid injections can provide immediate pain relief while also reducing inflammation in the afflicted joint. However, due to potential side effects and diminishing results with repeated injections, their use should be restricted.

Indeed, arthroplasty is another efficient treatment of OA. However, one million knee and hip joint replacements are done every year comprising more than US$27 billion in cost globally given the current impact, it is vital to develop novel treatments that alter disease progression instead of merely alleviating the pain or replacing the joints impacted entirely [5-7]. More importantly, this surgery risks permanent joint disability.

5.2. Bioengineering treatment and stem cell treatment

Bioengineering procedures aim to restore joint function and alleviate pain by repairing or replacing damaged joint tissues. Tissue engineering uses biomaterials like hydrogels and scaffolds to promote the growth and differentiation of chondrocytes, and joint resurfacing procedures involve making small holes in the subchondral bone or transferring healthy bone and cartilage tissue from a donor to encourage the growth of new cartilage and repair the damaged joint surface. While these approaches have shown promise in preclinical studies, their clinical efficacy and long-term results are still being evaluated.

Autologous Chondrocyte Implantation (ACI) is a new type of cartilage transplantation that includes extracting healthy cartilage cells from the non-affected portion of the patient's joint, cultivating them in a laboratory, and then implanting them into the injured area to encourage cartilage regeneration. To encourage the growth of new cartilage and repair the damaged joint surface, joint resurfacing procedures, such as microfracture surgery and osteochondral allograft transplantation, involve making small holes in the subchondral bone or transferring healthy bone and cartilage tissue from a donor. Instead of transplanting cartilage directly, utilizing stem cells' regenerative capacity to restore damaged joint tissues is a promising strategy for treating OA. For the treatment of OA, various stem cell types have been investigated, including MSCs (mesenchymal stem cells), which although previously extracted from bone marrow, a variety of other tissues, including adipose tissue, and synovial fluid can also be

DOI: 10.54254/2753-8818/21/20230872

used. They differentiate into chondrocytes and release substances that encourage the regeneration of cartilage and lessen inflammation. MSCs can be injected directly into joints or used in conjunction with biomaterials for tissue engineering purposes.

With the development of induced pluripotent stem cell (iPSCs) strategies, another type of assessing stem cells displays the advantages in the treatment of OA. Adult somatic cells can be reprogrammed to iPSCs, which can then be used to create new pluripotent cells, then differentiate into diverse cell types including chondrocytes. iPSC-derived chondrocytes can be employed as a cell source for ACI or other tissue engineering techniques promoting cartilage repair. Stem cell therapy remains a promising approach for treating OA. However, there are still challenges to be addressed, such as optimizing cell sources, delivery strategies, and safety profiles, before stem cell therapy can be widely used in clinical settings. Moreover, apart from novel bioengineering stems cell therapies, for example, new disease-modifying osteoarthritis drugs (DMOADS), can be used in addition to conventional treatment, and have the potential to provide more long-term and non-surgical solutions for OA by addressing underlying tissue damage and promoting joint repair. By addressing the underlying causes use of DMOADs can lead to continued improvements in joint function and pain relief, enhancing the quality of life for patients with OA. However, these approaches are still in clinical trials and may not be accessible or suitable for all patients.

5.3. Other non-pharmacological treatment

Non-pharmacological interventions, such as physical therapy, exercise, weight management, and the use of assistive devices, are also of use in improving the quality of life for people with OA. Regular exercise, including low-impact activities like swimming, cycling, and walking, can help maintain joint flexibility and muscle strength, ultimately reducing pain and stiffness. Weight management is crucial, as excess weight(obesity) puts additional stress on weight-bearing joints, exacerbating OA symptoms. Additionally, non-pharmacological interventions such as supplementing with glucosamine and chondroitin sulphate have also been used to treat OA symptoms, although their efficacy and safety profiles are still under investigation [4].

6. The outcome of osteoarthritis

Pain, stiffness, and limited mobility often lead to a lower quality of life in patients with OA. Without therapy, the progressive nature of OA may lead to worsening symptoms and further functional decline, negatively affecting daily activities and overall well-being. With therapy, many patients can manage their symptoms and maintain a relatively high quality of life. However, for some, especially those with advanced OA, conservative treatments may not provide adequate symptom relief and surgical options, such as joint replacement or joint fusion, may be considered to improve function and alleviate pain. Successful surgeries can significantly improve the quality of life for patients with severe OA, but they come with potential risks and complications [8-11].

7. Conclusion

Osteoarthritis is a widespread and debilitating joint disorder with a significant global impact. The disease's progression is primarily attributed to the breakdown of ECM components and the subsequent decline in chondrocyte function, leading to cartilage damage and inflammation. The ageing global population is expected to lead to an increased prevalence of OA, necessitating more effective treatment strategies. Current pharmacological treatments primarily focus on symptom relief and improving joint function, but pain medications when used long-term can cause deleterious side effects, and joint replacement surgeries carry inherent risks. Therefore, there is an urgent need for the further development of novel, disease-altering therapies that can both alleviate symptoms and address the underlying pathophysiology of OA. Emerging treatments, such as regenerative medicine techniques and targeted gene therapies, hold significant promise for the future management of OA. By better understanding the mechanisms involved in OA progression and exploring innovative therapeutic approaches, it is possible

DOI: 10.54254/2753-8818/21/20230872

to develop more effective interventions that could improve the quality of life for millions of people suffering from this debilitating condition.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230883

Review of antibody-drug conjugates in lymphoma therapies

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Abstract. Antibody-drug conjugates (ADCs) are a series of targeted therapeutic agents for cancer treatment and have succeeded in treating various cancers. Over the years, ADCs have revolutionized cancer treatment by offering new options to patients. These agents act as an alternative to traditional chemotherapy and radiation therapy, and many pharmaceutical companies have developed their own ADC drugs. Based on the available materials, this review discusses the fundamental concepts behind the design of ADC drugs for treating lymphoma. It provides a comprehensive analysis of various marketed ADC drugs from multiple perspectives.

Keywords: antibody-drug conjugates (ADCs), cancer therapy, combinatorial strategies, lymphoma.

1. Introduction

Lymphatic cancers, such as non-Hodgkin's inert lymphoma, can be challenging to treat. The most available lymphatic cancer treatment options are chemotherapy, radiotherapy, and immunotherapy, and neither of them are facing significant disadvantages. Although chemotherapy and radiotherapy are relatively effective treatment, the non-specificity always cause significant side effects, including but not limited to nausea and vomiting, fatigue, decreased appetite, changes in taste, hair loss, dry mouth, and constipation [1]. Despite the efficacy and relatively low cost of these treatments, they may cause great suffering for patients [2].

In 1913, German scientist Paul Ehrlich introduced the "Magic bullets", which aimed to provide efficient and targeted treatment using biotechnology [3]. The hybridoma technique was developed using the natural hybridization technique, leading to the creation of the first monoclonal antibodies. The launch of rituximab and trastuzumab in 1990 marked a breakthrough in cancer treatment, as it became possible to target antibodies to cancerous cells through specific binding, known as "Immunotherapy" [4]. However, despite decades of development, the efficacy of immunotherapy remains inconsistent, and its effectiveness is often limited by the patient's immune system [5]. Most patients with low immunity might not experience significant therapeutic effects from the treatment, and there is also a risk of immune inflammation [6]. On the other hand, with the first successful clinical trial of ADC in 1983 and approval of the first ADC drug—Mylotarg—by the FDA in 2000 [7], several companies entered the industry and developed their ADCs. Up to 2022, 14 ADCs have received market approval, and over 100 ADC candidates are in the clinical investigative stage [8].

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DOI: 10.54254/2753-8818/21/20230883

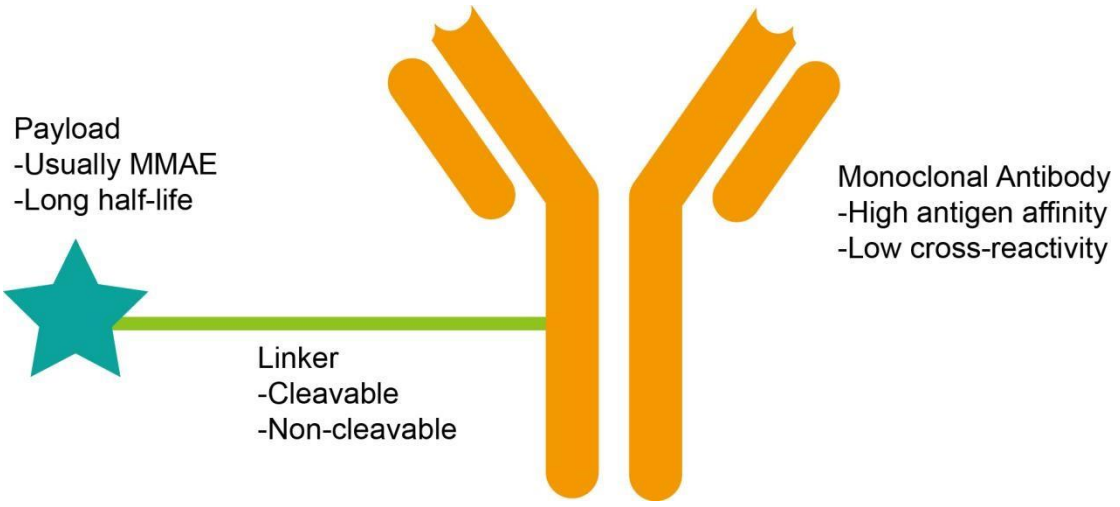

Figure 1. Components of ADCs.

2. ADCs structure

ADC drugs are consisted of three basic units, antibodies, payloads, and linkers.

2.1. Monoclonal antibody (mab)

Monoclonal antibodies (mAbs) play a crucial role in ADC therapy, as they can specifically recognize and attach to tumor cells' antigens, providing a targeted approach for cancer treatment. This specificity and affinity to surface antigens are essential for antibodies to connect to the corresponding cells. Unlike small-molecule therapeutics, which may fail to distinguish the healthy human cell from the tumor cells, ADCs, in this aspect, are pretty advantageous due to their explicitly recognizing ability to the tumor cells' antigens. For antibodies to connect to the antigen-correspond cells, the specificity and affinity to the surface antigens are essential [9]. Generally, it is difficult for drugs to distinguish a normal cell from a normal human cell since their surface antigen is identical. This consistency causes difficulties in choosing the targeted antigen when designing the drugs [10]. However, the invention of monoclonal antibodies and the discovery of tumor-specific antigens changed this in 1970, making it possible to target specific antigens in tumor cells [11].

There are mainly five classes of antibodies found in serum—lgM (Immunoglobin M), IgD, IgG, IgE, and IgA. Responsible for specifically binding to antigens, these antibodies act as the main structure of the ADC. Thus, when selecting monoclonal antibodies for ADC design, it is essential to consider their cross-reactivity and immunogenicity in patients [12]. lgG1 is the most frequently used antibody due to its simplicity in production and low clearance during circulation.

To minimize the risk of adverse immune reactions, humanized (Modified antibodies from non-human species) antibodies and human antibodies are preferred for most ADCs.

2.2. Cytotoxic drugs (payload)

Cytotoxic drugs are expected to possess high stability, weak immunogenicity, and a long half-life since their primary aim is to induce cell death. [13]. ADCs typically utilize microtubule-disturbing or DNA-damaging agents as their payload [14].

2.3. Linker

Since linkers connect the mAb and the payloads, their stability in circulation is essential. To ensure the release of the payloads at the proper time and place, the linker must be efficient enough to be cleavable in target tumor cells as it needs to release the payloads [15]. Linkers could be categorized into two groups-non-cleavable and cleavable—according to their payload release mechanisms [16]. Non-cleavable linkers are relatively stable in systemic circulation as they primarily degrade in lysosomes

DOI: 10.54254/2753-8818/21/20230883

[17]. In contrast, cleavable linkers, as they mainly depend on their physiological conditions, are less stable during systemic circulation [18]. For example, Mylotarg, the first FDA-approved ADCs, uses a linker to release its payload (ozogamicin) when exposed to acidic conditions, leading to severe adverse reactions in some patients.

3. ADCs mechanism of action

ADC drugs are administered into the body through intravenous injection, and bind to antigens on the tumor cell surface. The bound ADCs are then internalized by tumor cells, resulting in endosomes, where ADC molecules bind to Fc receptors (FcRns) in endosomes. The ADC molecule bound to the Fc receptor is re-transported outside the cell. As the pH rises, the ADC molecules separate from the Fc receptors. The ADC drug that is not attached to the Fc receptor is eventually retained in the endosome and transported to the lysosomal compartment, where it is degraded, leading to the release of the cytotoxic drug payload. However, when the ADC drug is injected into the body, there is a loss of ADC drug at each step from blood circulation to cellular internalization. Without sufficient cytotoxic molecules released into the cell, eventual apoptosis cannot appeal. Therefore, maximizing the efficiency of each step is crucial for the overall effectiveness of ADC therapy.

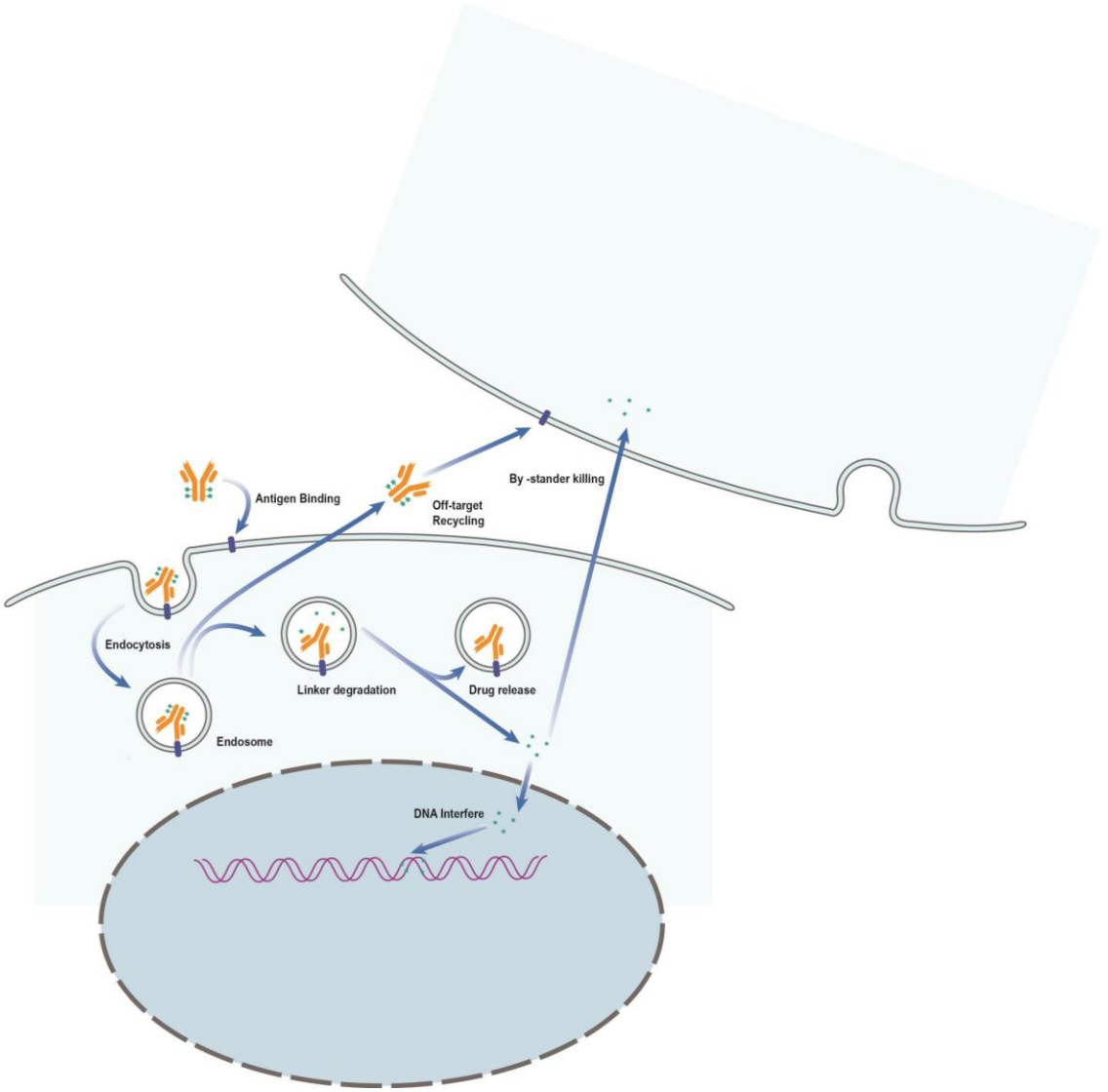

Figure 2. Mechanism of ADCs.

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4. Bystander killing

Bystander killing is a significant advantage of ADCs that use cleavable linkers. When the cytotoxic payload is released from the ADC molecule, it can diffuse out of the targeted tumor cell and kill neighboring cancer cells, regardless of whether they express the target antigen. This effect can increase the efficacy of the treatment by eliminating a larger area of the tumor. However, bystander killing can also cause damage to healthy cells, leading to unwanted side effects. To achieve bystander killing, the payload molecules must be able to cross the cell membrane. Thus, they should be nonpolar and neutral-charged to penetrate the cell efficiently. The cleavable linker must also be stable enough to retain the payload molecules in the ADC molecule during systemic circulation, but cleavable enough to release them when they reach the target tumor cells.

Antibody-Drug Conjugates in Clinics For Lymphoma.

Table 1. ADCs in clinics target for lymphoma therapies.

ADCs	Antibody	Linker	Payload	Target	indication	Approval Year
Loncastuximab tesirine (Zylonta)	Humanized IgG1	Val-Ala, Cleavable	SG3199 PBD dimer	CD19	diffuse large B-cell lymphoma, high-grade B-cell lymphoma.	20 December 2022, (EMA), 23 April 2023 (FDA)
polatuzumab vedotin (Polivy)	Humanized IgG1	Val–Cit, Cleavable	MMAE	CD79B	Diffuse large B cell lymphoma	16 January 2020 (EMA), 10 June 2019 (FDA)
Brentuximab Vedotin (Adcetris)	Chimeric lgG1	Val–Cit, Cleavable	MMAE	CD30	Relapsed/refractory Hodgkin lymphoma, systemic anaplastic large cell lymphoma	25 October 2012(EMA), 19 August 2011(FDA)

5. Loncastuximab tesirine

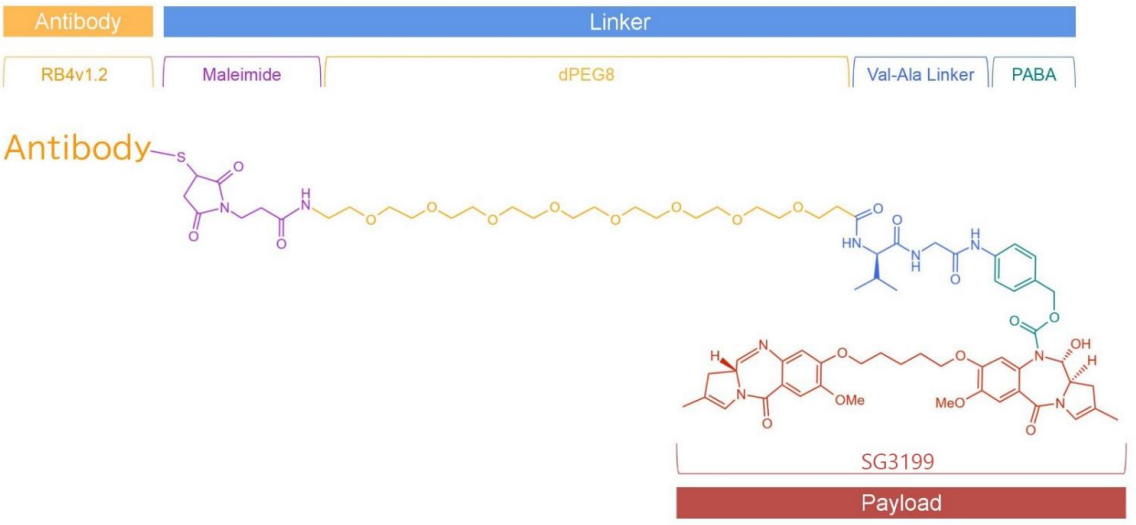

Figure 3. Molecule structure of Loncastuximab tesirine.

Loncastuximab tesirine (Commercial name: Zynlonta) is an ADC for relapsed or refractory large B-cell lymphoma treatment for two or more lines of systemic therapy, including diffuse large B-cell

DOI: 10.54254/2753-8818/21/20230883

lymphoma (DLBCL), but unspecified DLBCL evolved from low-grade lymphoma, and high-grade B-cell lymphoma [19, 20].

Known as the first and only ADC drug targeting CD19.Loncastuximab tesirine is composed of humanized RB4v1.2 mAb, Maleimide, dPEG8, Val-Ala protease-cleavable linker, PABA, and an SG3199 PBD (pyrrolobenzodiazepine) dimer alkylating agent [21]. SG3249 PBD dimer, knowns as Tesirine, SG3199 is the payload released from SG3249 [22]. SG3199 could retain picomolar activity in a panel of cancer cell lines, which is also known as one of the most commonly used and most cytotoxic payloads among ADCs [22]. In April 2021, FDA granted the accelerated approval of Loncastuximab tesirine. The approval is based on data from LOTIS-2, a large (n=145) phase 2 multinational, single-arm clinical study of Zynlonta for the treatment of adult patients with relapsed or refractory DLBCL following two or more prior lines of systemic therapy. Results from the trial demonstrated an overall response rate (ORR) of 48.3% (70/145 patients), which included a complete response (CR) rate of 24.1% and a partial response (PR) rate of 24.1%. Patients had a median time to response of 1.3 months and the median duration of response (mDoR) for the 70 responders was 10.3 months (inclusive of patients who were censored) [23]. However, the safety profile reports a LOTIS-2 study (N=145) with serious adverse reactions occurring in 28% of patients. The most common serious adverse reactions that occurred in ≥2% of patients were febrile neutropenia, pneumonia, edema, pleural effusion, and sepsis [24].

6. Polatuzumab vedotin

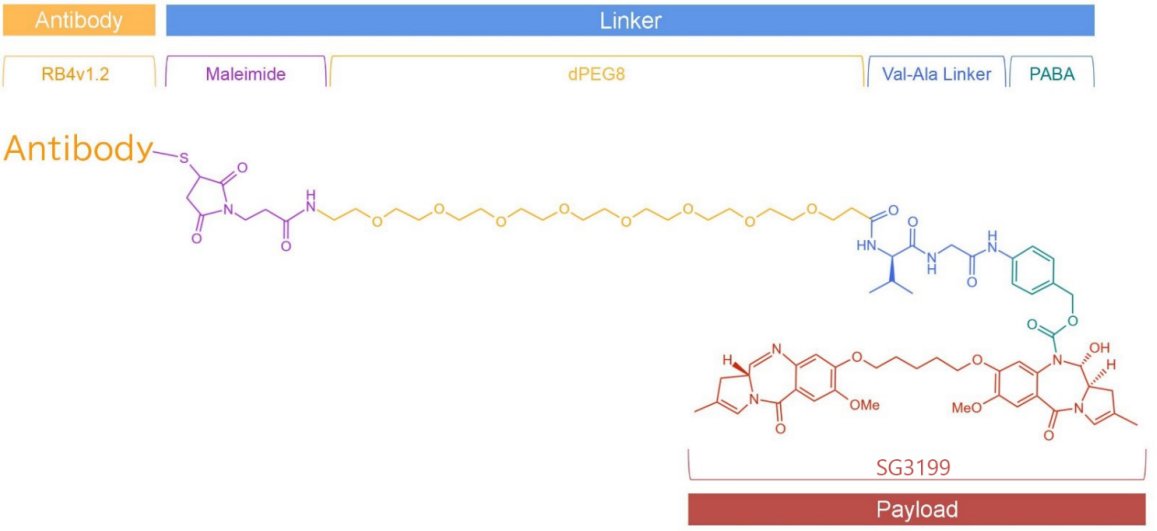

Figure 4. Molecule structure of Polatuzumab vedotin.

Polatuzumab vedotin (Commercial name: Polivy) is an ADC composed of an anti-CD79b mAb linked to tubulin inhibitor MMAE.

Polatuzumab vedotin was granted accelerated FDA approval on June 10, 2019 [25]. Polatuzumab vedotin is usually used to treat adult patients with relapsed or refractory diffuse large B-cell lymphoma, not otherwise specified, after at least two prior therapies with bendamustine and rituximab combined [25]. Polatuzumab vedotin could specifically bind to CD79b and cause the formation of endosomes. Once the lysosome protease cleaves the Linker, MMAE would be released. As one of the most common payloads among ADCs, MMAE could disturb the formation of tubulin polymerization and cause cell apoptosis [25-27[. The appearance of Polatuzumab vedotin also provides an advance in rituximab-cyclophosphamide-hydroxydaunorubicin-Oncovin-prednisone (R-CHOP) in Diffuse large B-cell lymphoma (DLBCL) therapy--polatuzumab vedotin in combination with rituximab, cyclophosphamide, doxorubicin, and prednisone (Pola-R-CHP), a front-line treatment. A clinical trial

DOI: 10.54254/2753-8818/21/20230883

reveals that the risk of disease progression, relapse, or death is lower among those who received Pola-R-CHP than among those who received R-CHOP [28]. However, the use of Pola-R-CHP is only in its place, notably, in selected patients [29].

7. Brentuximab vedotin

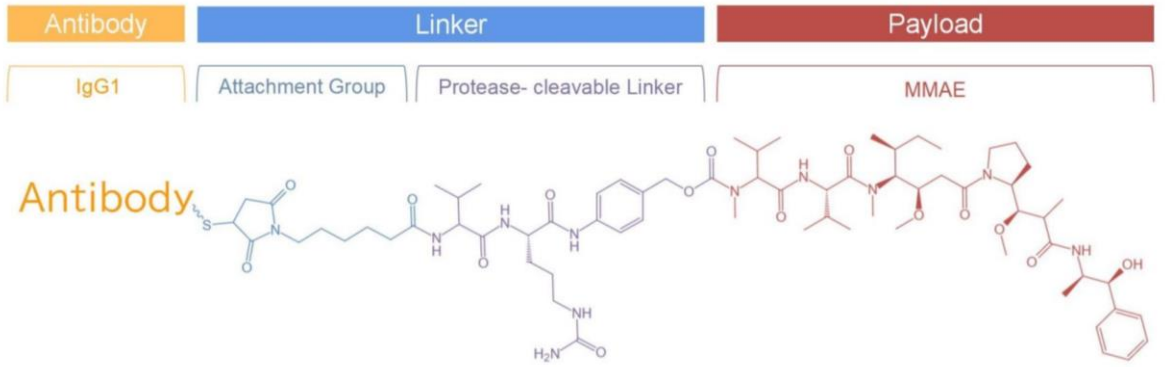

Figure 5. Molecule structure of Brentuximab vedotin.

Brentuximab vedotin (Commercial name: Adcetris) is composed of humanized lgG1 antibody (cAC10), maleimide attachment groups, protease-cleavable linkers, para-aminobenzylcarbamate pacers, and MMAE (payload) [30]. FDA approved Brentuximab vedotin marketing on August 19, 2011 for Hodgkin lymphoma and systemic Anaplastic large cell lymphoma (sALCL) treatment [31]. Brentuximab vedotin is approved for more than 65 countries for relapsed or refractory sALCL or relapsed or refractory classical Hodkin lymphoma therapies [32, 33]. Brentuximab vedotin applied a conditional cleavable valine-citrulline linker to maintain high stability in serum [34].

Through targeting and activating the CD30 on the tumor cells' surface, the brentuximab vedotin will be internalized and transported into the lysosomes, where the proteases cleavage linker will be decomposed into peptide and release MMAE (payload). The payloads released will further interfere with the microtubule construction and polymerization, which will eventually cause cell cycle arrest and apoptosis [35, 36]. The result obtained from first-generation ADCs, shows that only nearly 0.1% of ADC injections could reach their target, which shows the necessity of developing a DAR or potency of the cytotoxicity in the payload of ADC design [37, 38]. Adcetris improved both aspects by utilizing MMAE, a payload with significant cytotoxicity, as its payload. For more, Adcetris increased its DAR to nearly 4, which is significantly larger compared with the first-generation ADCs, like Mylortarg(gemtuzumab ozogamicin) with a DAR of two to three [39]. For patients who have once get treated with Brentuximab vedotin, retreatment of Brentuximab vedotin is often effective [40]. According to an encouraging result of a case series [Bartlett et al. 2010 [41]], Brentuximab vedotin exhibits a significant efficiency in retreatment tolerance. Based on this study, a phase II study about patients who previously experienced an objective response to brentuximab vedotin is ongoing. In March 2018, FDA approved Brentuximab vedotin coadministered in chemotherapy in adult patients who had previously untreated stage III or IV classical Hodgkin lymphoma [42]. Brentuximab vedotin is a treatment before Autologous Hematopoietic Stem Cell Transplantation in Hodgkin lymphoma and also as a consolidation of postautologous transplant in Hodgkin lymphoma [43].

8. Conclusions

As one of the most encouraging cancer therapy developments that ever appeared in human history, ADCs show great potential in overcoming the limitations of conventional therapies. Furthermore, there are tens of kinds of ADCs waiting for marketing approval. Though the current marketing ADCs lack variance toward a specific tumor, with the promising benefit from ADCs' potential market, hundreds of ADCs will be used in cancer therapies within decades.

DOI: 10.54254/2753-8818/21/20230883

However, there are still challenges brought by toxicities from complicated antibody and drug design. Further research and clinical application study is needed. In the near future, the clinical application of ADCs will be generalized, which will lead to the accumulation of more knowledge and boost the design of ADC drugs by scientists. As a result, various kinds of treatment options for cancer patients will be well-developed.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230888

The gut microbiome - an essential role for human health

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Abstract. The complex and vast ecosystem of microorganisms within the gut, known as the gut microbiome, plays an important role in regulating numerous biological systematics. From immune system function and metabolism to brain health and enteric nervous system regulation, dysbiosis or changes in its composition can have significant impacts on human wellbeing such as autoimmune diseases, metabolic disorders, neurological and psychiatric disorders, and enteric neuropathies. Fortunately, research has uncovered the potential for targeting and manipulating the gut microbiome as a strategy in managing a wide variety of human ailments. By focusing on promoting a healthy balance within the gut's microbial community, experts believe that theoretically curative treatments could arise related to the reduction/prevention of many diverse conditions which affect overall health. Through advances in understanding this crucial mechanism, we may be able to develop more effective therapies to promote optimal human health. As genetic sequencing technology continues expanding our knowledge in the field, future breakthroughs await those who patiently continue studying the fascinating world of the gut microbiome.

Keywords: gut microbiome, therapeutic strategy, human diseases.

1. Introduction

The complex web of intestinal microbes, or gut microbiome, is recognised as a main critical actuator of human health. Numerous microorganisms, including bacteria, viruses, fungus, and other microbes, are found in abundance throughout the human gastrointestinal system and play crucial parts in a variety of biological processes. The gut microbiota significantly influences how nutrients are absorbed, how energy is metabolised, how well the immune system works, and other vital physiological processes. Type 2 diabetes, obesity, inflammatory bowel disease, as well as many neurological and mental illnesses, have all been linked to dysbiosis in humans. The gut microbiota governs a massive range of biological variants functions and has a significant influence on human health. For instance, controlling immunological function is one of the most important functions of gut microorganisms. The formation and differentiation of immune cells, immune system activation, and the creation of immune-modulatory chemicals are all significantly influenced by the gut microbiome [1]. Furthermore, it has been exemplified that the gut microbiota has a substantial impact on controlling metabolic processes in the human body, including nutrition absorption and metabolism. Numerous metabolic diseases such as diabetes (type 2), obesity, and onset cardiovascular disease, have been related to changes in the gut microbiota makeup. Additionally, recent studies have demonstrated that the gut-brain axis has an

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DOI: 10.54254/2753-8818/21/20230888

impartial relationship to gut microbiota. which is a system of two-way communication between the CNS and the digestive system. Through neurotransmitter production, immune system modulation, and stress response regulation, the gut microbiota affects activity of the brain and possible behavioural habits. Dysbiosis has been previously connected to a number of mental and neurological conditions, which can include autism spectrum disorder, sadness, and anxiety. The gut microbiota may affect cognitive function, memory, and learning, according to growing research [2]. Research into the intricate interactions between humans and their gut bacteria is expanding quickly. It has a tremendous deal of potential for the creation of innovative treatments for several ailments. Understanding and treating a variety of human diseases may undergo a revolutionary change by the cause of research into the effects of the gut microbiome on immunological, metabolic, and cognitive functions. The gut microbiome is very dynamic and flexible, and it may be influenced by many things, inclusive of nutrition, drugs, and environmental exposures, according to recent studies. The gut microbiota is now understood to have a remarkable role in determining general health and wellbeing. It is becoming more and more obvious that targeting the gut microbiota is advantageous for generating innovative therapeutics for a wide range of human ailments as research into it continues to grow. How the gut microbiota affects human health and illness is reviewed in this article. It focuses on immunological, metabolic, and cognitive function and talks about how targeting the gut microbiota may be used therapeutically. In addition to discussing the potential of dietary interventions aimed at manipulating the use of the gut microbiota as a powerful method of illness prevention and treatment in people, the article will examine the most recent research findings on the inter-relationship occurring through the gut microbiome and a diversification of diseases, including autoimmune diseases, metabolic disorders, neurological and psychiatric ailments and disorders. This essay's main objective is to provide an in depth argument about the crucial role played by the gut microbiome in human health and ailments as well as to emphasise the possibility for therapeutic targeting of the gut microbiome.

2. Analysis of the gut microbiomes

2.1. The gut microbiomes' pertinence to human physiology

A much-discussed collective population of microorganisms, better known as the gut microbiome, has recently gained attention for its critical role in human health regulation. Present within the human gastrointestinal tract are trillions of varied microbes that include viruses, fungi and bacteria, all of which possess their own imperative functions regarding diverse biological mechanisms. In fact, many studies have associated significant breaches and dysfunctions in the composition of the internal microbiota to numerous afflictions such as type 2 diabetes, obesity, irritable bowel syndrome, etc., not to mention neurological and mental ailments.

The gut microbiome's efficacy on human bodies is mainly evident with regards to the governing of immunity functioning; playing a vital part in the differentiation, activation and formation of immunomodulatory molecules by immune cells. Research has suggested that their presence serves as a protective barrier against autoimmune conditions, hypersensitivities, allergies and other closely related diseases. A newer study explained how these microbial populations also play a crucial function in preventing type 1 diabetes, which is defined as an increasingly commonplace chronic autoimmune disease arising from the destruction of beta cells responsible for insulin production. Consequently, it appears inevitable that targeting said microbiomes may present an opportunity for effective new treatments for such medical conditions [3].

In addition, the prevalence of the gut microbiome in metabolic processes, namely digestion and absorption of fibers/carbohydrates, ensuring appropriate energy distribution and body mass control along with maintaining healthy insulin levels, can't be overlooked either. Various disorders arise when there is a notable disruption in the microbiome composition, likewise being seen in cardiometabolic conditions such as diabetes of the second class and corpulence. According to a more contemporary paper, the utilization of artificial sweeteners - interchangeably utilized for sugars - affects the microbiome's balance leading to glucose intolerance and further metabolism incidents [4].

DOI: 10.54254/2753-8818/21/20230888

Furthermore, the connection between and the gut-brain network and central nervous system (CNS) is worth highlighting particularly as it concerns the peculiarities of cognitive functioning, behavior and temperament. As findings suggest, modifications in the microbiome integrity can alter brain activities, linked to expressing states of anxiety and depression, for example, as well as autism spectrum disorders. Interestingly, a recent investigation illustrated that infusing the faecal microbiota of depressive patients into germ-free mice led to behaviors resembling depression too, clearly indicating the potency of such an association in the genesis of mental illnesses [5].

To top it off, it's important to note the relationship between the gut microbiome and the enteric nervous system (ENS); the latter possessing intricate nerve networks controlling physiological processes such as gastric movement, secretions, et cetera. Studies suggest that changes in the microbiome can modify the development and activity of the ENS while also having implications on gut motion and secretion. Additionally, a relevant article has implied that problems relating to the enteric nervous system like enteric neuropathies are likely due to imbalances in the gut microbiome, hence hinting toward its potential therapeutic value for such maladies [6].

Conclusively, the indisputability of human wellbeing shows the importance of gut microbiomes, considering its involvement in various bodily functions such as immunity, metabolism, brain physiology and the enteric nervous system. Dysbiosis - signifying a loss of stability - and imbalance in gut microbiota composition have long been connected with divers afflictions, ranging from metabolic flaws to psychiatric issues. Thus, modern science strives to fabricate novel remedies influenced by this organism assortment, given its potential to improve human health conditions overtime.

2.2. Effects from the gut microbiome communities

The gut microbiome is a highly agile and adaptive community that can be profoundly impacted by an array of variables, such as diet, medicines, and environmental exposures. Investigational evidence has revealed that this particular microbiome onsets important roles in both human health and illness and is now recognized as an integral factor for wellbeing.

This microbiome forms an indispensable element of the digestive process and helps to break down indigestible complex polysaccharides while ferments fibers to generate short-chain fatty acids (SCFAs). SCFAs serve as energy sources for the intestinal epithelium and other tissues within the body. Witnesses furthermore show that the total makes up of the gut microbiome directly correlates with the intake and implementation of nutrients from diet. To elaborate, a presence of distinctive bacterial taxa has been linked to improved absorption of dietary fiber to increase production of SCFAs and better glucose metabolism [7,8].

One of the pivotal roles that gut microbiome plays is in regulating the integrity of the intestinal epithelial barrier. Acting as both a physical and biochemical barricade between the luminal contents of the gut and underlying tissues, this intricate mechanism is essential for overall gut health. Dysbiosis within the gut microbiome can advance an increased permeability and inflammation, subsequently resulting in conditions such as celiac disease, irritable bowel syndrome, and inflammatory bowel disease [9].

Beyond maintaining healthy gut function, the gut microbiome also plays an influential part in systemic metabolism regulation. This entails modulating host metabolic processes via the production of numerous metabolites including SCFAs and secondary bile acids which influence physiological characteristics like energy balance or glucose & lipid metabolism. Studies show that changes in gut microbiome are in cahoots with elevated risks of metabolic disorders such as obesity, non-alcoholic fatty liver diseases and diabetes (type 2) [10].

Another critical context in which gut microbiome is responsible for is overseeing the immune system of host organisms. The micobiota interact with local and systemic cells of the host's immune system which depends upon the microorganisms residing within the gut. New researches reveal interaction requires influence over these interactions. Immune cell development and functionality - such as B-cells, T-cells, and antigen-presenting cells – are significantly influenced by the gut

DOI: 10.54254/2753-8818/21/20230888

microbiome. Consequently, influences on the body's ability to respond to pathogens and defend against autoimmune disorders occurs [11].

Gut-brain communication is regulated by the gut-brian connection mediator ie; vagus nerve where neurotransmitters travel extending its influencing areas beyond localized domains. Gut-microbes have the intriguing properties to produce many sorts-of neurotransmitters and signaling molecules such as serotonin or GABA. These molecules aid brain activity and role manifestation cerebrally resulting in behavioral outputs. Pathways of neurological and psychiatric origination are linked-back to variations observed in gut microbiomes pertaining to depression, anxiety and autism spectrum disorders [8]. The gut-brain communication is an essential parameter to achieve a balanced bodily function. Variations in the composition of the said microorganisms can be linked back to some important neurological like autism or physiological disorders like obesity [12].

At last, newly-emerging research has demonstrated that the gut microbiome may also play an indispensable role in cancer's advance and formation. The microbiota are apt at collaborating with the host's cells, both internally in the bowels and around the body overall, while investigations have proven that disparities in the gut microbiome can result in adjustments to the host's susceptibility to cancer. For example, discrepancies in the gut microcosm have been bonded to a heightened risk of colorectal cancer and hepatocellular carcinoma [13].

Consequently, the maintenance of a prospering gut microbiome is critical for personal well-being and health on the whole. The gut microbiome assumes an imperative part in the organization of the intestinal epithelial fence, general metabolism, the immune system, as well as intellectual intuitiveness. changes in the gut microbiome have been attached to a widespread array of illnesses, including inflammatory bowel malady, metabolic disorder, and cancer. Subsequent examination is required to understand the multifaceted relations between the gut microbiome and human well-being, so that appropriate therapeutic treatments may be created to tackle a variety of disorders.

2.3. Methods in improving the gut microbiome

Preserving a thriving gut microbiome is indispensable for long-term health. Investigations have verified that diet and lifestyle are essential influencing elements when constructing gut microbiota composition, diversity, and function. Therefore, several suggestions assist in endorsing a healthy gut microbiome.

First, consuming various plant-based foods is essential for sustaining a vibrant gut microbiome. One study located that those who depended upon a plant-based diet had an extensive gut microbiota compared to people that ate an animal-based diet. On top of this, fiber-filled foodstuff such as entire grains, fruits, and vegetables, have been indicated to raise the abundance of favorable gut bacteria [14].

In order to keep a healthy gut microbiome, one must limit the intake of ultra-processed foods and added sugars. As found in an article by Suez et al., consuming a high-sugar, high-fat diet resulted in a considerable decrease in gut microbiota diversity as well as a heightened presence of destructive bacteria. Additionally, Menni et al. also discovered that those who consumed diets heavily composed of ultra-processed foods had less varied gut microbiotas when compared to individuals eating mainly whole food-based meals [15]. One can potentially amplify his/her gut wellbeing by incorporating fermented foods into the diet, for instance kefir, sauerkraut and yogurt, as these items contain live bacteria which may assist in increasing the abundance of advantageous gut bacteria [16]. Moreover, recent and relevant studies indicate that partaking of fermented foods is collaterally correlated with improved gut microbial heterogeneity. To ensure optimal gut health, one must also get proper rest and properly manage stress levels as sleep deprivation leads to a diminution of gut microbiota diversity while an undue rise in deleterious bacterium is attributed to chronic stress [17].

Ultimately, consuming probiotics and prebiotics may be advantageous for sustaining a thriving gut microbiome. Prebiotics are nonabsorbable carbohydrates that advocate the propagation of beneficial bacteria in the gut [18]. A study conducted by Holscher et al., found that ingesting a fiber supplement containing prebiotics led to an increase in helpful gut bacteria. Probiotics are live organisms with therapeutic effects when taken regularly in suitable amounts. Studies have demonstrated that

DOI: 10.54254/2753-8818/21/20230888

probiotics successfully restore the balance of the gut microbiome by escalating the abundance of salubrious bacteria, while lowering the growth of deleterious ones. For example, research discovered that a daily mixture of Bifidobacteria and Lactobacilli for four weeks heightened the amount of useful bacteria - such as Bifidobacteria and Faecalibacterium prausnitzii-whereas reducing the presence of dangerous microorganisms, including Clostridium difficile and Streptococcus spp [19].

In conclusion, it is crucial to maintain a healthy and balanced gut microbiome for overall well-being and health. The composition, diversity, and function of the gut bacteria are significantly affected by daily lifestyle choices such as diet and sleep patterns. To foster a healthy gut flora one should adopt certain habits like including a variety of whole foods in their menu preferences while reducing processed food and added sugar intake, incorporating fermented products into their eating habits, managing stress levels, prioritizing quality slumber, and using prebiotics and probiotics supplements that act on nurturing beneficial gut microbes.

3. Conclusion

The gut microbiomes takes on a crucial role in the assimilation and breakdown of diverse nutrients, such as complex carbohydrates, essential minerals, and vitamins. Additionally, it supports the maintenance of a healthy immune system while regulating both metabolism rate and influencing mental health through the gut-brain axis mechanism. Numerous scientific studies show that alterations within this microbiota can inflict different medical conditions on an individual such as obesity, diabetes, and various mental health disorders. The importance of maintaining sufficient quantities of healthy gut microbiota via balanced dietary intake and lifestyle modifications is emphasized in this article to promote overall well-being. Nevertheless, our coherent knowledge about the conjunction between the gut microbiota and other bodily systems has its limitations since only the relationship between the gut microbiota and the brain-gut axis has undergone extensive research so far. Therefore, more research into understanding these relationships with other modes of functionality like the endocrine and immune systems is required, among others. Another challenge we face is that standardized data collection methodologies regarding gut microbiota research have not been agreed upon yet; hence results remain open to interpretation across trials, bearing variation in composition analysis methods affecting generalizability. Lastly, the quantity, structure, and complexity of gut microbiota varies significantly between individuals further illustrating the ambiguities presently surrounding related conclusions based on robust inferences. The advancement of research in this field should center on constructing validated protocols for gut microbiota investigation and exploring the connection between gut microbiota and other internal components. Furthermore, longeritudinal studies with significant participations are required for strengthening our comprehension regarding how lifestyle aspects like nutrient intake, physical activity, and pressure influence gut microbiota characteristics and functions. Moreover, the potentials of therapeutic interventions for the breakthrough of the gut microbiota are exceedingly alluring for exploration. Evidently, probiotics, prebiotics and other alimentary supplements hold cooperative traits to optimise gut microbiota formation and accelerate beneficial health outcomes. Apart from that, Faecal Microbiota Transplantation (FMT) also became a promising cure for various intestinal issues such as repetitive Clostridioides difficile contamination, inflammatory bowel deteroriation and irritable bowel syndrome. In spite of many remaining doubts and complications, the possibility of therapeutical intervention modulating the gut microbiota carries magnitude of welfares concerning wellness policies.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230889

Overview of benzene and exploration of benzene structure

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Abstract. Benzene is a hydrocarbon the simplest aromatics. But the search for benzene's structure has been tortuous. This article mainly introduces the basic properties of benzene. From the physical properties of benzene to the chemical properties of benzene to the uses of benzene. In this work, we also summarize the process of scientists' exploration of benzene structure and introduce the background of benzene. Of all the structures of benzene, the Kekule structure is the most recognized structure. We have proved the correctness of the Kekule structure by the product of the ozonation decomposition reaction of o-xylene. However, the Kekule structure has some limitations, the search for benzene structure is still ongoing, and we are still trying to find ways to better describe the structure of benzene. So we also sum up some modern theories about the structure of benzene. In addition, benzene is a very common chemical raw material. The article also lists some chemical products made from benzene.

Keywords: benzene, Kekule formula, Hybrid orbital theory.

1. Introduction

The discovery of the molecular structure of benzene in middle school textbooks has been described by the anecdote of Kekule's "wonderful" dream, and some chemistry history textbooks refer to the "structure theory of benzene" as an example of chemical intuition [1]. High school textbooks are limited in length, present students with little knowledge, and attribute great scientific discoveries to an accident, which always feel unconvincing. As we all know, the revelation of scientific laws is a slow accumulation process, a process in which quantitative changes cause qualitative changes. Kekule's revelation of the structure of benzene should also be a process of quantitative change causing qualitative change. What is the process of discovery of Kekule's "dream"? What about the large π bond in the benzene ring in modern valence bond theory?

2. Background

2.1. Discovery of benzene

In the early 19th century, the United Kingdom and other European countries were widely used in urban lighting gas. Gas production left a kind of oily, smelly, viscous liquid for a long time no one. As shown in Figure 1, English scientist Faraday was the first to become interested in this oily liquid. After five years of research, in 1825, he isolated a new hydrocarbon from the liquid. Faraday called it a "heavy carbon compound of hydrogen" and determined its experimental formula CH by analysis. In 1834,

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DOI: 10.54254/2753-8818/21/20230889

German chemist Michilrich named it benzene, and then French chemist Gerard determined its molecular formula as C6H6 [2].

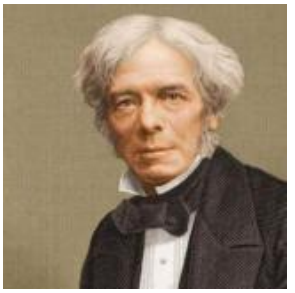

Figure 1. Faraday

2.2. Source of benzene

The light tar produced in the coking process of coal contains large amounts of benzene. This is how benzene was originally produced. The generated coal tar and gas are passed through the washing and absorption equipment together, and the coal tar with a high boiling point is used as the washing and absorption agent to recover the coal tar in the gas. After distillation, crude benzene and other high boiling point fractions are obtained. Industrial-grade benzene can be obtained by refining crude benzene. The purity of benzene obtained by this method is relatively low, and the environmental pollution is serious, and the technology is relatively backward [3].

Small amounts of benzene are present in crude oil, and the extraction of benzene from petroleum products is the most widely used preparation method [3].

At 500-525℃, 8-50 atmospheres of pressure, a variety of boiling points between 60-200℃ aliphatic hydrocarbons, by platinum-rhenium catalyst, through dehydrogenation, cyclation into benzene and other aromatic hydrocarbons. After the aromatic hydrocarbon product is extracted from the mixture, benzene is separated by distillation. These fractions can also be used as high-octane gasoline [4].

Steam cracking is a process for producing alkenes from low molecular alkanes such as ethane, propane or butane, and petroleum components such as naphtha and heavy diesel. One of its byproducts, cracked gasoline, is rich in benzene and can be fractionated into benzene and other components. Cracked gasoline can also be mixed with other hydrocarbons as additives to gasoline.

About 40-60% of benzene in cracked gasoline, but also contains diolefin and styrene and other unsaturated components, these impurities in the storage process easy to further react to the formation of polymer gum. Therefore, first through the hydrogenation process to remove these impurities and sulfide in the cracked gasoline, and then the appropriate separation to obtain benzene products.

2.3. Toxicity of benzene

Because benzene is volatile, it disperses easily when exposed to air. Inhalation or skin contact with large amounts of benzene into the body can cause acute and chronic benzene poisoning in humans and animals. Some studies have reported that benzene poisoning is partly due to the formation of phenol from benzene in the body.

Benzene has a paralyzing effect on the central nervous system, causing acute poisoning. In severe cases, headache, nausea, vomiting, confusion, loss of consciousness, coma, convulsions, etc., and in severe cases, death due to central system paralysis. A small amount of benzene can also cause drowsiness, dizziness, rapid heart rate, headache, shaking, confusion, confusion and other phenomena. Ingesting food containing too much benzene can cause vomiting, stomach pain, dizziness, insomnia, convulsions, rapid heart rate and even death. Breathing 20‰ benzene vapor for 5-10 minutes can be fatal.

Long-term exposure to benzene can cause great damage to the blood, causing chronic poisoning. Causes neurasthenic syndrome. Benzene can damage bone marrow, reduce the number of red blood cells, white blood cells, and platelets, and cause chromosome aberration, which can lead to leukemia, and even aplastic anemia. Benzene can cause excessive bleeding, which suppresses the immune system and

DOI: 10.54254/2753-8818/21/20230889

allows disease to take hold. Studies have reported that the incubation period of benzene in the body can be as long as 12 to 15 years.

3. The discovery of benzene molecular structure

3.1. Conjectures on the structure of benzene

Benzene was discovered in 1825, and for decades its structure was unknown. Everything about benzene showed that its molecules were very symmetrical, but at the time it was hard to imagine how six carbon and six hydrogen atoms could be arranged in perfect symmetry to form a stable molecule.

Since 1825, many chemical researchers have speculated that benzene should have its own special structure based on the different properties of benzene and aliphatic compounds found at that time, and should propose new structure or new valence bond explanations for benzene.

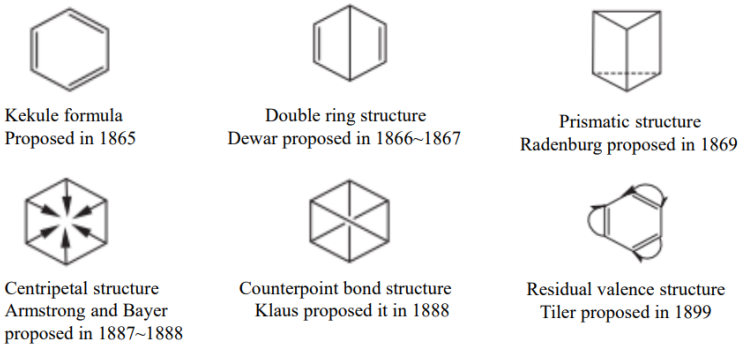

Figure 2. Several molecular structures of benzene

Figure 2 shows the molecular structure formula proposed by different scientists in different ages. In 1857, the German chemist Kekule proposed the theory of tetravalent carbon. In 1858, he proposed that benzene molecules have a ring structure. Suppose a benzene molecule's six-carbon chain is connected to each other in a ring, and each carbon is connected to a hydrogen, so that the six hydrogens occupy the same positions. In 1890, at a ceremony commemorating the 25th anniversary of the benzene ring structure, he described his scientific discovery as a "dream" inspired by a moment's inspiration.

The double-ring structure was proposed by Dewar and was historically known as Dewar benzene, which is now known to be a reactive cycloolefin - dicyclo-2, 5-hexadiene [2.2.0].

The prismatic structure is called prism alkane, which is also very active.

Armstrong and Baeyer proposed the centripetal formula and argued that: in benzene molecules, the fourth valence of each carbon atom points to the center of the ring and is not connected to other atoms. This is called the central bond, and the six central bonds balance each other, making the combined energy of each bond a potential force. The central bond does not exist in aliphatic compounds, so the aromatic properties can be considered to be due to the special symmetrical arrangement of the fourth valence of the carbon atoms in the ring.

Tiler's theory of residual valence holds that the double bond in the structural formula of residual valence cannot use all the single valence, so a part of the unused valence is left, which is called residual, and the residual valence combines with each other to form a new bond. According to this assumption, the bonds between C and C in the benzene ring are roughly equal. There is no difference between single and double bonds, and the covalence of every two adjacent carbon atoms in the six carbon atoms are combined with each other, becoming a new system.

The covalent or central bond theory proposed new solutions to the structural formulations of benzene. Essentially, they are in general agreement with the modern methods of describing the structural formulations of benzene. But at that time, it was impossible to elucidate the nature of the central bond and covalent, and there were no suitable experimental methods for further confirmations. Therefore,

DOI: 10.54254/2753-8818/21/20230889

these two structures contrary to the classical valence bond theory have not been universally accepted by chemists.

As shown in Figure 3 and Figure 4, modern studies have shown that rosin and dewar bene are also related to benzene. It is proved that benzene can be changed into benzene and dewar bene by light excitation. Although benzene and Dewar bene conform to classical valence bond theory, this structure is inconsistent with the related properties of benzene.

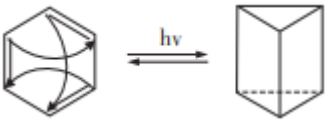

Figure 3. Benzene is converted to prism

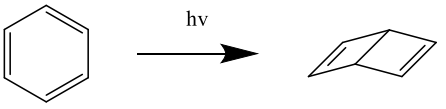

Figure 4. Benzene is converted to dewar benzene.

As shown in Figure 5 and Figure 6. Kekule's formula reveals that benzene can have only one unary substitution and three binary substitutions.

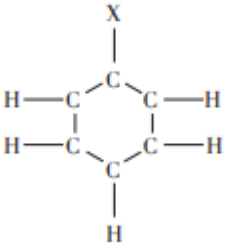

Figure 5. One unary substitute of benzene

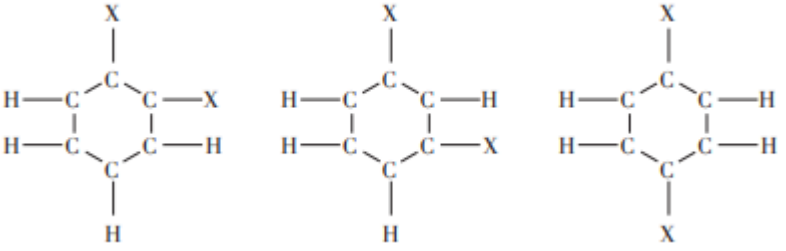

Figure 6. Three binary substituents for benzene

After many studies, it is found that the number of isomers of benzene substitutes is always consistent with Kekule's ring structure, and Kekule's form has achieved great success. According to the hexagonal ring structure of benzene, each carbon only needs to remove the trivalent, and the remaining one is combined with each other to form three double bonds. Every single bond has a double bond, so each carbon becomes quadrivalent and meets the theoretical requirements of the quadrivalent carbon. However, Kekule's formula has two major disadvantages: first, it does not explain why benzene molecules, since they have double bonds, do not normally react with the addition of reagents of unsaturated hydrocarbons; Secondly, according to such a structure, there should be two isomers of the

DOI: 10.54254/2753-8818/21/20230889

ortho-binary substitution of benzene, but this is inconsistent with experience, and various tests have shown that there is only one ortho-binary substitution.

To solve this contradiction, Kekule proposed an alternative explanation, which was to assume that the double bonds in benzene were not fixed in position, but could move back and forth so fast that it would not be possible to tell the difference between single and double bonds. This was the so-called oscillating double bond theory [2].

3.2. Confirmation of modern experiments

In the 20th century, due to the progress of theoretical physics and physical methods, people verified the structure conjectures of benzene.

Modern physical methods such as x-ray method and spectrum method have proved that the benzene molecule is a planar regular hexagon configuration. The bond Angle of benzene and the bond length of the carbon-carbon bond in benzene have been determined.

As shown in Figure 7, the scanning tunneling microscope gave people their first glimpse of benzene, and an image of benzene molecules was published in the *journal Chemistry Today*, making the hexagonal structure of benzene molecules clearly visible to people.

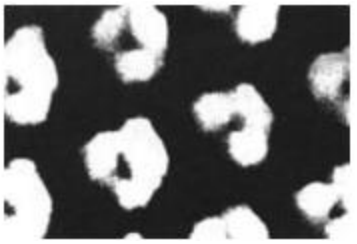

Figure 7. Images of benzene molecules were obtained using a scanning tunneling microscope

3.3. Theoretical explanation of benzene molecular structure

It is an accepted fact that the shape of the benzene molecule space is hexagonal. Is the Kekule formula of benzene molecule alternating single and double bonds correct? This has been a nagging topic.

As the research progressed, it was found that three compounds, butanedione, acetone aldehyde and glyoxal, could be formed by the interaction of o-xylene and ozone.

As shown in Figure 8, this suggests that benzene and benzene derivatives may indeed have two structures with different double bond arrangements, as Kekule suggested, because if there is only one structure, either one should produce only two compounds. However, benzene can not discolor bromate water and acid potassium permanganate solution, and it proves that benzene does not have the characteristic of unsaturated hydrocarbon, that is, it does not have the structure of carbon-carbon double bond, so people have doubts about the Kekule formula.

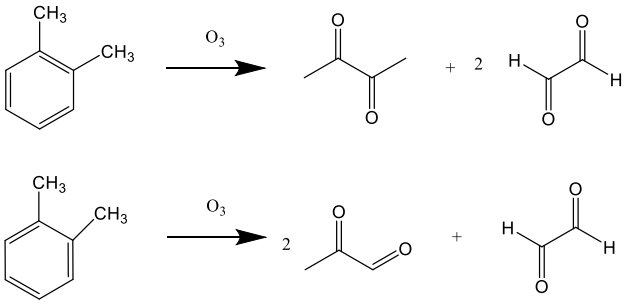

Figure 8. Two ozonolysis products of o-xylene

So what is the structure of benzene?

DOI: 10.54254/2753-8818/21/20230889

With the development of chemical theory, some chemists have put forward some theoretical explanations for the structure of benzene, which seem to accord with the relevant chemical properties of benzene. The typical ones are as follows:

(1) Hybrid orbital theory

Hybrid Orbital Theory was proposed in 1931 by Pauling L and others on the basis of the valence bond theory. The theory of hybrid orbitals holds that the electrons of the six carbon atoms in the benzene molecule overlap each other with sp2 hybrid orbitals to form six σ bonds of carbon and carbon, and each of them overlaps the 1s orbital of hydrogen atom with 1 sp2 hybrid orbital to form six σ bonds of carbon and hydrogen. Because sp2 hybridized, the bond Angle is 120. And all six carbon atoms and six hydrogen atoms are connected to each other on the same plane, forming a regular hexagonal structure. The six carbon atoms on the benzene ring do not participate in the hybrid 2p orbital. They are perpendicular to the ring plane, their sides overlap each other and form a closed large π bond, which is evenly distributed above and below the ring plane. The large π bond of benzene makes the property of benzene more stable than that of unsaturated hydrocarbons, and it is generally not easy to occur the addition reaction of general unsaturated hydrocarbons [4].

(2) Molecular orbital theory

Molecular orbital theory, also known as molecular orbital theory (Molecular orbital theory) or MO method, was proposed by American chemist R.S. Mulliken (R.S. Mulliken) and German physicist F. Hundt (F. Hundt) in 1932. The molecular orbital theory states that six p orbitals are linearly combined into six π molecular orbitals, three bonding orbitals and three antibonding orbitals. The ground state is six p electrons in pairs filling three bonding orbitals, so all of the low energy bonding orbitals, which are all lower energy than the original atomic orbitals, are full of electrons, so the benzene molecule is stable, and the system is low energy. The large π bond of the benzene molecule can be seen as the result of the superposition of three π-bonding orbitals. After the superposition of orbitals, the electron cloud density between each adjacent carbon atom is equal, and the bond length of the benzene carbon-carbon bond is completely average [5].

(3) Resonance theory

As shown in Figure 9, resonance theory is a theory of molecular structure formulated by the American chemist L.C. Pauling in 1931 for the discussion of molecules that cannot be described in terms of valence bond structures. Resonance theory holds that benzene mainly resonates between the two Kekule structures, and the energy of the whole system is reduced. Resonance makes the carbon-carbon bond neither single nor double, and the six bonds are the same, and the benzene ring is stable. Resonance makes the hydrogenation energy of benzene 121.8kJ·mol-1 lower than the theoretical calculated hydrogenation energy of 1, 3, 5-cyclohexatriene, which is called the resonance energy of benzene. It is because of the resonance energy that the benzene ring is more stable and can replace the addition [2].

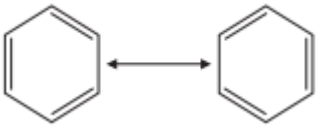

Figure 9. Resonance of benzene

(4) Molecular structure of modern benzene

In recent decades, some people have raised questions about "the π electron of benzene is delocalized" and "the delocalization of the π electron in benzene makes benzene stable". The new idea is that the symmetrical hexagonal structure of benzene depends only on the σ electron, and that the π system of benzene does not favor a delocalized "aromatic hexagon", but rather a structure with three localized π bonds. Cop-per et al. put forward the spin coupling valence bond theory in "Electronic Structure of benzene molecules" published in 1986. According to this theory, the two localized Kekule structures are a pair of "electron tautomers", which represent the microstructure of the compound molecule and cannot be separated. From a microscopic point of view, compounds can be multi structural, that is, a compound

DOI: 10.54254/2753-8818/21/20230889

may have several microscopic structures. We usually talk about the molecular structure is the macro-structure of molecules. A compound molecule can only have one macro-structure, so the macro-structure is a balanced structure mixed with several micro-structures. Benzene is actually a balanced mixture of two microscopic structures (Kekule structure). Spin coupling valence bond theory differs from resonance theory, which holds that benzene has a variety of molecular structures, and benzene is a mixture of structures. According to the spin coupling valence bond theory, benzene has only one molecular structure, but one pair of internal microstructure is inseparable [6].

The structure of benzene and its expression have been discussed for more than 170 years. So far, no satisfactory conclusion has been reached. The study of benzene molecular structure is far from over, and it still needs to be explored by aspiring chemists. Now people commonly used benzene molecular structure formula is: one is the Kekule formula; The other is represented by a regular hexagon with an inner circle representing the π electron cloud in the benzene ring as a whole.

4. Products made from benzene

4.1. Friedel-Crafts alkylation reaction

As shown in Figure 10, Friedel-Crafts alkylation is the connection of an alkyl group to an electron-rich benzene ring or derivative catalyzed by Lewis acid. First, halogenated hydrocarbons are ionized by Lewis acid to form a carbocation electrophilic body. The aromatic ring then attacks the carbocation, forming a carbon-carbon bond and a new carbocation intermediate. Finally, cyclic deprotonation restores aromaticity, while Lewis acid is regenerated.

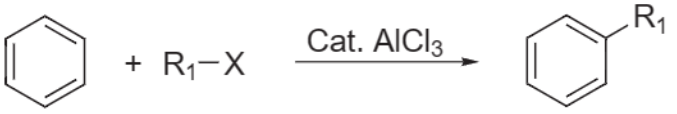

Figure 10. Friedel-Crafts alkylation

4.2. Friedel-Crafts acylation reaction

The electrophilic substitution reaction of an aromatic compound with an acyl halide or anhydride catalyzed by a protic acid or Lewis acid, such as aluminum trichloride, is a modified electrophilic substitution reaction. Figure 11 shows the Acylation mechanism.

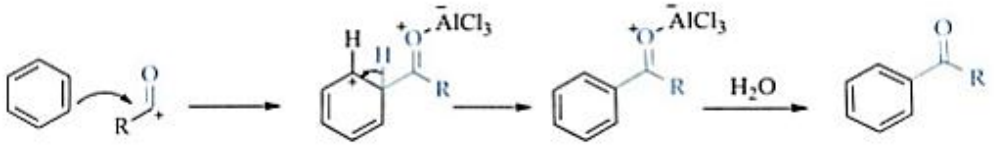

Figure 11. Acylation mechanism

Benzene is a very important chemical raw material. Many chemical products are prepared from benzene.

5. Epilogue

The benzene molecule is a kind of highly unsaturated molecule, but it is easy to replace and difficult to add. This is a time to create new ideas, and to create new ideas requires establishing a logical relationship between evidence and conclusion. Many guesses have been put forward about the molecular structure of benzene, but the Kekule formula is proven to meet the requirements based on the fact that benzene has only one unary substitution and three binary substitutions. The evidence of ozonation and reduction

DOI: 10.54254/2753-8818/21/20230889

of ortho-xylene-produced products proved that the Kekule formula did not fully meet the requirements, and scientists made new theoretical hypotheses for the structure of benzene.

The structure of benzene and its expression has been discussed for 170 years. Although various hypotheses have been put forward, no satisfactory conclusion has been reached. This is still a problem that needs further investigation. Research is continuing on the structure and properties of benzene.

6. Conclusion

This paper mainly introduces the basic properties of benzene and its background, and tells about the structure of the benzene exploration process. From different periods of different scientists put forward the structure analysis. The Kekule structure formula is widely recognized as one of the structures. However, the Kekule structure formula also has some limitations, so from the analysis of modern research theory, the structure of benzene still needs to be explored. At the same time, benzene as one of the most basic chemical raw materials, according to Friedel-Crafts reaction can produce many chemical products.

In the future, the exploration of benzene structure will continue, there will be more appropriate theories to describe the structure of benzene, and in chemical synthesis, benzene as a basic chemical raw material, there will be more synthesis routes using benzene as raw material will be developed.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230890

Research on healthy human sleep circadian rhythms

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Abstract. The exhaustion of humans during the day is the primary motivation for this paper. Circadian Rhythm is a necessary system, which is the 24-hour cycle that is part of humans' inner clock that controls and organizes the essential functions and processes in our body. A significant example of circadian rhythms is the cycle of sleep-wake. Sleep is an inalienable part of human life (humans will die for sure if they stay awake for 10 years). However, the length of sleep will decrease based on human's age. The older human is, the less sleep they will require. However, circadian rhythm can greatly impact dietary intake and physical activities and is fundamentally impacted by brightness and sleep-inducing substances. And this paper will introduce how to scientifically set up the circadian rhythm, and how to maintain a healthy circadian rhythm by utilizing those 4 main factors. So that reduces the exhaustion for humans dyscalculia from the circadian rhythms; and implies a healthy, positive, and efficient society. And the goal for this paper is that advising human maintain a consistently daily life which can greatly reduce exhaustion.

Keywords: circadian rhythms, sleep, brightness, time, exhaustion.

1. Introduction

Exhaustion is an unalienable human emotion or feeling. Being exhausted is a negative feeling. Especially in the early morning, a decent amount of people still constantly feel drowsy during work for example, less effiency on finishing daily routine, and causing healthy conditions such as insomnia, endocrine dyspraxia…(Eng Min J 2018 Feb; 219(2):38-40); the majority of students are sleepy during class (Harms M. Individual differences in tolerance to shift work: a review. Ergonomics. 1993;36(1-3):101-9.). Phenomenon above demonstrate the study of sleep is mandatory since the unhealthy circadian rhythms will disrupt our daily life or even directly impact our health. Therefore, the purpose of this paper is to explore a certain way to help the majority of people reduce their exhaustion in real life and increase their length of wakefulness. As a result, sleep and circadian rhythms pop up immediately. Humans spend one third of their lives sleeping; however, is their sleep ideal and useful? which leads to today's core and centripetal question: "What is the scientific way to set up the circadian rhythm so that it keeps humans awake and fulfilled with energy as much as possible". A better circadian rhythm not only reduces exhaustion and increases wakefulness, but also promotes a healthier daily routine and Hormone release. Therefore, this paper investigates the method of setting a scientific circadian rhythm. This research contributes to human health and the normal operation of society.

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DOI: 10.54254/2753-8818/21/20230890

2. Analysis of setting scientific circadian rhythms

First, this section will present the ideal time for sleeping depending on age and health conditions.

Table 1. the ideal sleeping hour for ages of people.

Age	Average time for sleeping (hrs per day)
Newborn babies (0-3 months)	14-17
Infants (4-11 months)	12-15
Toddlers (1-2 years)	11-14
Preschoolers (3-4 years)	10-13
School-age children (5-12 years)	9-11
Teenagers (13-17 years)	8-10
Adults (18-64 years)	7-9
Elder (over 65 years)	7-8

An overall pattern is that older people require less time to sleep [1]. Since children's bodies require more hours of sleep to develop and function properly [2]. The advantages of sleep can be summarized as: restoration (remove metabolic end products that damage and inhibit their brain's foundation function); memory processing (SWS associated with hippocampal replays encoded neural patterns that consolidate humans declarative memory), and Dreaming (irrelevant but exists) [3, 4]. Besides the length of sleep, the quality of sleep is also an unalienable section. Sleep quality is based on how hard it is for a person to fall asleep and remain asleep, as well as how many times they wake up during a night. There is no certain relationship between quality and quantity of sleep. There is no possible way to replace the lack of sleep quantity with the quality of sleep, and vice versa. The Sleep Need Index, also known as SNI, was applied to self-check whether one needed more sleep or not. The formula: sleepiness/sleep duration is calculated as a number range, taking SNI value 0.26 as the judgment value; if it is above 0.26, then people definitely need more sleep, and vice versa [5].

The circadian rhythm is the 24-hour cycle that is part of the body's internal clock that runs in the background to carry out essential functions and processes. One of the most important and well-known circadian rhythms is the sleep-wake cycle. The circadian rhythm fundamentally depends on hormonal signals from the hypothalamus or process C and S, as well as the brightness captured by the suprachiasmatic nucleus, known as SCN, a brain area directly above the optic chiasm [6]. Brightness greatly impacts circadian rhythm since brightness is the main signal of what time it is. If the SCN doesn't detect light, the pineal gland is free and automatically produces melatonin, which is a type of sleep-inducing substance that will make humans feel drowsy. For instance, exposure to light during the night can suppress melatonin secretion, and increase wakefulness [7,8]. While a precise 24-hour circadian rhythm is found in most organisms, it isn't universal or unique; organisms living in the high arctic or high antarctic don't experiment with daytime all year, which generally maintain certain circadian rhythms that close to 24 hours, such as the penguins and polar bears whose lives are in the south pole and north pole [9]. Although there are still a decent amount of organisms that reside in the dark biosphere, and these may exhibit rhythmic physiology, the dominant rhythm is not going to be circadian. Therefore, brightness has a huge impact and is an important tool for scientifically resetting the circadian rhythm. In short, sleep-wake Homeostasis, also known as Process S, is the accumulation of sleep-inducing substances in the human body. In simple terms, the longer people stay awake, the more those sleep-inducing substances will accumulate in the brain. The amount of sleep-inducing substances directly impacts wakefulness in humans (possibly as a potential way to impact circadian rhythms by controlling

DOI: 10.54254/2753-8818/21/20230890

the production of sleep-inducing substances). One neurochemical indicator of a sleep-inducing substance is adenosine, which will under-stimulate body function and sensitivity [10].

Besides those scientific and fancy techniques, there are still other factors that can greatly impact humans' circadian rhythms. Dietary intake and physical activity also impact circadian rhythm. Food is one of the external synchronizers of the human peripheral clock, which has the same concept as brightness: the primary role of circadian rhythm is to entrain the organism to environmental cues. Therefore the anticipation of food availability and food choice can influence the judgment of circadian rhythm. There is no doubt that limiting dietary intake at certain times during the day will not fundamentally but greatly impact behavior and physiology, which indirectly impact the circadian rhythm [11]. Physical activity or exercise induces physiological changes, such as body temperature and hormonal signaling, that affect peripheral circadian clocks through sympathetic activation and glucocorticoid release. For instance, the elevation of body core temperature will cause a higher heart rate and become an input to the circadian pacemaker gradually impacting circadian rhythms [12].

Now back to the original topic of this paper, start with the interference of light and brightness. Based on the intervention of a cycled light system in the ICU room, which simulates the real world for supporting patients' circadian rhythms, a result that most satisfies the circadian rhythms. Most patients reported that their sleep was worse in the ICU with intervention than at home. This makes sense because as mentioned before circadian rhythms are extremely dependent on people. However, the conclusion that can be drawn is that as long as people follow natural circadian rhythms and consist of them, therefore they will end up with a decent circadian rhythm [13].

Table 2. An intervention room that artificially for acting as a natural world. The illumination levels are based on time.

Light scenes in the intervention room	Time	Illumination levels in lux in horizontal plane
1	7-8 am	58
2	8-10 am	615
3	10-10:30 am	450
4	10:30 am - 1 pm	330
5	1-3 pm	210
6	3-5 pm	450
7	5-6 pm	330
8	6-7 pm	210
9	7-8 pm	81
10	8-8:45 pm	58
11	8:45-9 pm	30
12	9-9:15 pm	12
13	9:15-9:30 pm	8
14	9:30 pm- 7 am	2

As usual, as long as humans are in tune with their circadian rhythms, they don't need to worry about homeostasis. Because there is indeed a balance between the production and accumulation of sleep-

DOI: 10.54254/2753-8818/21/20230890

inducing substances and the destruction of sleep-inducing substances. Dietary intake can greatly affect circadian rhythms due to the different nutritional compositions and timing of meals in humans. Therefore, it is necessary to formulate a healthy diet according to the specific conditions unique to each individual, so as to ensure a healthy sleep rhythm. For example, substances such as caffeine, alcohol, or melatonin can significantly affect circadian rhythms. This article recommends careful consumption and never becoming addicted to any of these foods. It would be better if the meal time was consistent so that the body could form its own work and rest habits and adapt. Also, exercising is another good habit that is beneficial to human circadian rhythms. Therefore, this article suggests that people can do high-intensity exercise and vigorous exercise in the morning and low-intensity exercise in the evening. High-intensity exercise increases heart rate and body temperature and, most importantly, delays melatonin production. This helps humans stay awake during the day.

3. Conclusion

There is indeed a more general way of helping humans reduce exhaustion, and increase their efficiency as a whole. As a result, there are four main factors: dietary intake, activity, brightness, and sleep-inducing substances, which tremendously impact circadian rhythms. However, this paper is originally for humans as a whole instead of a certain group of people, which makes that too vague to actually "rescue" and "reduce exhaustion". In the future, it is hoped that future studies can expand the scope of the study, which can provide more detailed and reliable results. For example, hire testers and a controlled experiment was conducted; separated into three levels of intake groups: over intake, less intake, and normal intake. So that comparing data and ANOVA which will provide us result. As well as utilize animals experiment such as mice; the advantages of animal experiment are easier and more flexible for controlling and processing the experiment; and exploring more factors which provides a more specific and accurate result.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230891

HIF-1α inhibitor PX-478 for cancer therapy and other fields

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Abstract. Hypoxia-inducible factor 1-alpha (HIF-1α) is a crucial transcription factor for tumor growth and progression. PX-478, a small molecule HIF-1α inhibitor, has been extensively studied for its potential as an anti-cancer agent in preclinical studies. This paper provides an overview of the current status of PX-478 in cancer treatment, with a focus on its application in treating pancreatic ductal adenocarcinoma (PDAC), lung cancer, breast cancer, glioma, and other kinds of cancer. This article discusses the mechanisms of action, pharmacokinetics, and pharmacodynamics of PX-478, as well as the findings of preclinical and clinical studies conducted to date. The evidence suggests that PX-478 has promising potential as a therapeutic agent for various cancer types. However, further research is needed to fully comprehend the compound's potential benefits and limitations and its optimal clinical application.

Keywords: HIF-1α, PX-478, cancer therapy, combination treatment.

1. Introduction

It is widely known that cancer disease is the second leading cause of death and morbidity all over the world. It has been reported and estimated that cancer kills nearly 10 million people annually and this number is increasing year by year [1]. Due to the unique tumor microenvironment, the effect of many anti-cancer drugs and therapy in cancer treatment and prognosis is very poor and limited, especially in the PDAC, where the five-year survival rate is less than 10% [2]. Additionally, some tumors can evolve during the treatment and secrete some chemicals to alter their microenvironment, reducing their sensitivity to the therapies and promoting tumor aggravation.

Hypoxia, a characteristic feature in the tumor microenvironment, has been found in most solid tumors [2]. Under hypoxia, HIF-1α will translocate to the nucleus and then bind with HIF-1β to regulate the expression of various downstream genes, which play a non-substitutable role in the construction of the tumor microenvironment, such as angiogenesis, extracellular matrix remodeling, and cancer-associated fibroblasts formation [3]. These contribute to tumor progression, metabolic, metastasis, and even evocation from the immune system, which has been tightly associated with poor therapeutic effects and prognosis [2, 3].

PX-478 has shown great power in the treatment of various cancers by inhibiting the HIF-1α at multiple levels, including decreasing mRNA, reducing translation, and promoting degradation [4]. These inhibitions occurred in both hypoxia and normoxia and are independent from pVHL and p53 [4]. In previous studies, it has shown promising achievements in the alone treatment or in combination with other therapies for some cancers with high HIF-1α expression, such as pancreatic, prostate, lung

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DOI: 10.54254/2753-8818/21/20230891

cancer et al [4]. Recently, scientists have found its great potential in the treatment of Atherosclerosis and diabetes mellitus. Therefore, this paper will review the application of PX-478 in cancer treatment and discuss its prospects in other emerging fields.

2. Cancer therapy

Hypoxia is always associated with therapy-resistant and poor prognosis in cancer treatment by promoting cancer cell proliferation and metastasis [3]. Recent studies have reported that it contributes to tumor resistance to radiotherapy, chemotherapy, and immunotherapy in various cancer, especially in PDAC, and breast cancer [3]. In previous research, PX-478 has been demonstrated significant efficacy in cancer treatment by inhibiting HIF-1α at multiple levels [5]. Current studies are thus focused on the combination treatment of PX-478 with other therapies, which has been shown to significantly improve treatment outcomes in various types of cancer. This section aims to summarize the powerful effects of PX-478 in enhancing the anti-tumor activity of other therapies and briefly explain the underlying mechanisms.

2.1. Alone treatment

PX-478 has shown great power in treating cancer, especially solid tumors with high level of hypoxia, such as PDAC, ESSC, breast cancer, and lung cancer. It has been reported that can inhibit tumor growth and induce cell apoptosis by inhibiting G2/M transition, EMT, and angiogenesis for cancer treatment in vivo and in vitro [6]. In a study, the Administration of 20 mg/kg PX-478 resulted in a remarkable reduction of the median primary volume of NSCLC and SCLC by 87% and 99% respectively, and delayed their metastases [7]. Additionally, PX-478 single treatment appears to have the ability to induce and enhance immune response. Inflammatory factor COX-2 and immunosuppressive factor PD-L1, directly targeted by HIF-1α, were overexpressed in ESSC and associated with poor survival rate and prognosis [5]. Administration of PX-478 alone significantly suppresses the expression of PD-L1 and COX-2 and inhibits the tumor growth of ESSC in vivo and in vitro [6].

2.2. Combination treatment

Hypoxia and HIF-1α are involved in slow-proliferation stem-cell-like phenotype cell transformation, angiogenesis promotion, microenvironment remodeling, and metastasis augmentation, which all further induce therapy resistance [3]. Therefore, targeting HIF-1α to reduce tumor resistance to chemotherapy, radiotherapy is a promising strategy and has been extensively researched in recent studies [3]. Additionally, with immune therapy emerging, PX-478 also has been shown that can enhance the immune response when combined with immunotherapy.

2.2.1. Combination with radiotherapy. Hypoxia is highly associated with resistance of radiotherapy. On the one hand, cancer cells exposed to radiation in low-oxygen environments tend to resist cell death because there are fewer DNA radicals produced as a result of the reduced formation of ROS and DNA damage [3]. On the other hand, it can activate HIF-1α and downstream signaling pathways to promote angiogenesis and stromal recovery [5].

PX-478 was discovered that can enhance the sensitivity of prostate cancer cells to radiotherapy under hypoxia and normixa by prolonging the phosphorylation of H2AX histone which is positive for DNA damage [9]. Additionally, PX-478 has been demonstrated that can improve the radiosensitivity in Panc-1, C6 glioma, UMSCCa10, and HN5 squamous cell lines by blocking HIF-1α dependent microenvironment remodeling and stomal vascular support [8]. Treatment with PX-478 in hypoxia one day before radiation provided a consistent radiation sensitization enhancement of 1.42 [8]. In pancreatic xenografts of Panc-1, SU.86.86, and CF-PAC-1, PX-478 enhanced the antitumor effects of fractionated radiation, whether combined with gemcitabine or not [10]. Additionally, 5 days of GY radiation combined with PX-478 treatment made 56.6% and 80.9% maximum tumor regression respectively in Panc-1 pancreatic cancer xenografts [10].

DOI: 10.54254/2753-8818/21/20230891

2.2.2. Combination with chemotherapy. Combination therapy of many first-line chemotherapeutic drugs with PX-478 for cancer treatment has been widely reported in recent studies and shows great efficiency in many types of cancer, especially in PDAC, breast cancer, and lung cancer.

Gemcitabine (Gem) is one of the most widely used chemotherapeutic drugs for advanced pancreatic cancer. However, due to the high degree of drug resistance in PDAC which is the most hypoxia solid tumor, gem is often ineffective In PDAC treatment [2]. PX-478 has been reported that can significantly enhance the gem effect of inhibition of tumor growth and promotion of Immunogenic cell death (ICD) [2]. During ICD, dying cancer cells express a variety of signals, known as damage-associated molecule patterns to activate immune cells such as dendritic cells, macrophages, and T cells, which can recognize and attack other cancer cells [2]. In recent research, PX-478 has been demonstrated that can increase the antitumor effect of gem via inducing ICD in PDAC by upregulating P-elF2a [2]. In the xenograft model, immune-competent mice were vaccinated with Panc02 cells which were treated with Gem and PX-478 before [2]. The mice were then injected with Panc02 cells on the other flank for 1 week. The survival rate of the immunized group has significantly increased (5/8 alive) compared with the non-immunized or Gem-alone immunized group. Additionally, in spleen and tumor tissues, CD3+ and CD8+ cytotoxic T cells have been detected significantly increased [2]. In vitro, expression of CRT, HMGBI, and ATP, three of the hallmarks of ICD were highly increased in the combination treatment group. Combination treatments also have been shown to promote maturation and increase the phagocytosis activity of dendritic T cells [2].

Reactive oxygen species (ROS) play a complex role in cellular signaling and are implicated in the etiology of cancer. A moderate quantity of ROS aids in the development and growth of tumors, whereas excessive level of ROS can destroy cancer cell DNA, leading to cell death [11]. Arsenic trioxide (As2O3, ATO) is a ROS inducer that is frequently applied to treat a variety of malignancies, including lymphoma, and leukemia [11]. However, the ATO alone treatment in PDAC has been demonstrated that only had a minimal anti-tumor effect. HIF-1α has been reported that can partially inhibit ROS production under hypoxia [11]. Lang et al. recently have shown that PX-478 can significantly enhance the antitumor effect of arsenic trioxide by promoting apoptosis of cancer cells induced by excessive ROS [11]. Additionally, they found HIF-1α lessoned ROS independent of the mitochondrial pathway but through FOXO1/SESN3 pathway in PDAC (Panc-1 and BxPC-3 cells) [11]. Similarly, Dichloroacetic acid, a potential drug in oncology, and PX-478 have been shown to have synergistic effects in various cancer cell lines, including lung, colorectal, cervical, breast, brain, and liver cancers [12]. Additionally, ROS generation and apoptosis have been proven that plays important roles in this synergism [12].

Recent research has reported that miRNA also played a key role in the development of drug resistance in tumors [13]. Li et al found that Survivin, a protein inhibiting apoptosis, is significantly upregulated in TNBC by downregulating miRNA-494 in TNBC under hypoxia, which is associated with drug resistance [13]. Docetaxel (DTX), inhibiting the growth and division of cancer cells by interfering with the microtubes, is commonly used to treat various types of cancer, including breast cancer [13]. PX-478 has been shown that significantly decrease the resistance of TNBC to DTX through HIF-1α/miR-494/Survivin signaling pathway on both MB-231 and MB-468 cell lines [13]. Additionally, in the previous study, hypoxia was shown that can reduce the apoptosis of colorectal carcinoma (CRC) cells induced by oxaliplatin (OXA) [14]. Xu et al. recently revealed that HIF-1α/miR-338-5p/IL-6 feedback loop appears to be responsible for this. MiR-338-5p is significantly downregulated in HCT116 and HCT8 colorectal cancer cell lines. PX-478 administration can suppress this loop and enhance the cytotoxic effects of OXA on CRC [14].

Glioma is the most common primary malignant brain tumor [15]. Hypoxia and HIFs influence glioma development and survival by regulating angiogenesis, glycolytic metabolism, and treatment-resistant [15]. Therefore, targeting HIF-1α in glioma treatment appears to be a possible therapy. In a statical survey, ferroptosis was found to be associated with malignancy progression and drug resistance in 1750 glioma cases [15]. Sulfasalazine (SAS), which is commonly used to treat rheumatoid arthritis, has recently been shown to have anticancer properties in a variety of

DOI: 10.54254/2753-8818/21/20230891

malignancies, including gliomas, via triggering ferroptosis and inhibiting SLC7ALL [15]. Sun et al. revealed that hypoxia enhances the resistance of glioma cells to SAS-induced ferroptosis via the P13K/AKT/HIF-1α/SLC7ALL pathway [15]. In this research, PX-478 and SAS have been proven to have a synergistic effect on anticancer activities both in vivo and in vitro [15].

In addition to the cancer xenograft model that was directly cultured in the lab, patient-derived models were employed to investigate the potential of PX-478 combined with other drugs. Ryu et al. have reported that PX-478 single treatment or its combination with neratinib (20 mg/kg) significantly suppresses tumor growth in cases of trastuzumab-exposed HR-/HER2+ patient-derived breast cancer xenograft models and VEGF was downregulated [16].

2.2.3. Combination with immunotherapy. Recent advances in immunotherapy have shown significant progress in treating various types of cancer. However, hypoxia remains a major challenge for immunotherapy, as it triggers the activation of HIF-1α and its downstream pathway, which is crucial for the construction of the immunosuppressive microenvironment. Therefore, targeting HIF-1 for cancer treatment seems to be a promising strategy to dismantle the immunosuppressive network of the microenvironment.

Immune checkpoint inhibitors (ICI) are a popular therapy by blocking certain checkpoints on immune cells, allowing them to attack cancer cells more effectively [5]. However, the effectiveness of ICI treatment with NSCLC is not significant which may associate with low T-cell infiltration [5]. From previous research, it has become more and more clear that epithelial-mesenchymal transition (EMT) reduces TILs in the tumor microenvironment to inhibit anti-tumor immunity. Luo et al. has recently revealed that HIF-1α is crucial in inducing EMT with LOXL2 as an important bridge molecule and appears to decrease the quantity of TILS via promoting hypoxia-induced EMT [5]. In this research, ICI anti-PD-I synergized with PX-478 and got a remarkable result in NSCLC treatment [5]. Compared with signal treatment, combination treatment significantly increased tumor growth regression. Large increases in CD4+ and CD8+ T cells, IFN-γ production, and granzyme B were observed [5]. Additionally, Kheshtchin et al. have reported that in the 4T1 breast cancer model, PX-478 significantly enhances the anti-tumor effect of cell-based vaccination [13]. Co-administration of PX-478 with antigen-based DC vaccination resulted in total tumor regression in 50% of mice, as well as a significant increase in survival [13]. These findings show that inhibiting HIF-1α with PX-478 is a promising therapeutic for boosting anti-tumor immunity.

3. Other fields

In addition to its inhibitory effect on tumors, PX-478 has shown great potential in other diseases in recent research.

Hypoxia commonly occurs in adipose tissue due to the tissue's underdeveloped vascular system and the excess expansion of adipocytes to store fat [17]. Instead of promoting angiogenesis, ECM will accumulate abnormally under hypoxia in adipose tissue, causing extensive tissue fibrosis and resulting in dysfunction [17]. According to research by Sun et al., PX-478 treatment successfully inhibits the high-fat diet (HFD)-induced activation of HIF-1α in adipose tissue but did not affect weight gain for mice with a chow diet [17]. The treatment strengthens energy expenditure and increases resistance to metabolic parameter deterioration caused by HFD [17]. Furthermore, a reduction of fibrosis and inflammatory infiltrates were observed in the adipose tissue of mice treated with PX-478 [17].

Atherosclerosis is also significantly influenced by HIF-1α. Increased hypoxia is known to occur in atherosclerotic lesions, which cause inflammation, plaque development, and vascular remodeling [18]. Villa-Roel et al has demonstrated that PX-478 inhibits HIF-1α and its target genes in ECs that are associated with lipid and fatty acid catabolic pathways and reduces atherosclerosis in chronic mouse model injected with AAV-PCSK911 [17]. Additionally, the reduction of plasma cholesterol levels by 69% and 30% respectively, and prevention of diet-induced weight gain are observed in C57BL/6 and ApoE-/- mice, showing that PX-478 may be a promising drug in atherogenic treatment [18].

DOI: 10.54254/2753-8818/21/20230891

In type 2 diabetes development, pancreatic β cells undergo expansion to maintain normoglycemia, but prolonged metabolic overload leads to β cell dysfunction [19]. According to IIegems et al., metabolic overstimulation in mice pancreatic islets led to a hypoxic phenotype [19]. In mouse islet organoid PX-478 was shown to be able to restore normal insulin production in response to glucose [18]. Administration of PX-478 to db/db and STZ-induced diabetic mice caused a reduction in blood glucose levels and an improvement in β cell function, which suggests the potential therapeutic effects of PX-478 in treating diabetes [19].

4. Conclusion

PX-478 has demonstrated significant efficacy in the treatment of various types of cancer with high hypoxia, especially in PDAC, breast cancer, and lung cancer. In addition to its alone treatment, its use in combination with other therapies has yielded promising results in vitro and in vivo. It can enhance the activity of radiotherapy in several xenograft models. In terms of chemotherapy, it can enhance the anti-tumor effect of several first-line chemotherapeutic drugs, such as gem, ATO, DTX, and neratinib in various cancer treatments by inducing ICD, ROS, and ferroptosis. Additionally, when combined with immunotherapy, it can enhance the immune response, increasing the expression of cytotoxic T cells, cytokine, and other immune factors. However, despite its remarkable potential, there is still a need for further investigation to fully comprehend the underlying mechanisms of PX-478. Additionally, more clinical trials, animal studies, and in vivo experiments are necessary to determine the optimal utilization of PX-478 and explore its broader range of applications. Beyond its impact on cancer treatment, recent studies have found its great potential in the field of diabetes and atherosclerosis. However, the mechanism behind these remains limited, which also needs to be further researched.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230893

The influencing factors for depression in US adults: Data from the NHANES(2009–2018)

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Abstract. In today's world, as society puts more and more pressure on people, the incidence of depression is also increasing. Depression can have a number of serious harms, not only affecting an individual's emotional and psychological state, but also having a serious impact on quality of life, work and personal relationships. To find the causes of depression, this paper will analyze the contributing factors of depression by analyzing data from U.S. adults in NHANES(2009-2018), a series of cross-sectional, complex, multi-stage surveys conducted by the CDC(Centers for Disease Control and Prevention). Some variables were obtained from the questionnaire -- sleep status and smoking status. The results included age, sex, education, marital status, smoking status, obesity, and some of the diseases associated with CRD. This study found that sleep disorders had the largest OR value, Education level and smoking status have the least relationship with CRD because of the lowest OR value.

Keywords: depression, pressure, sleep, Arthritis, coronary heart disease.

1. Introduction

Depression is more than just sadness; it is a mood disorder that can cause lasting feelings of sadness and loss of interest [1], accompanied by changes in somatic and cognitive aspects that significantly impact individuals' functioning [2]. In recent years, the prevalence of depression has been increasing globally, including in the United States. Understanding the factors that contribute to the development of depression is crucial for effective prevention and treatment strategies.

In the US, the prevalence of depression has reached alarming levels. From 2015 to 2020, there has been a rising trend in depression rates, with a prevalence of over 9% in 2020. This poses a significant public health concern, as depression not only causes personal distress but also has wide-ranging societal implications.

Depression has a profound impact that extends beyond individual suffering. It affects relationships, work productivity, and overall quality of life. Individuals with depression often experience difficulties in maintaining relationships, impairments in occupational functioning, increased healthcare utilization, and higher rates of mortality. Additionally, depression is associated with an increased risk of developing other physical and mental health conditions, such as anxiety disorders, substance abuse, and cardiovascular diseases.

Contrary to popular belief, depression is not solely caused by a chemical imbalance in the brain. Research has shown that it is influenced by a combination of factors, including mood regulation, genetic

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DOI: 10.54254/2753-8818/21/20230893

vulnerability, and life events [3]. Similarly, the relationship between anxiety, depression, and sleep quality is not solely due to the feelings of depression and anxiety but also has a genetic component [4].

The purpose of this paper is to systematically review and analyze the various factors that contribute to the development of depression in adults in the United States. By doing so, we aim to contribute to the growing body of knowledge on depression and provide insights into effective prevention and intervention strategies.

2. Methods

The data is from the NHANES, a series of cross-sectional, complex, multi-stage surveys conducted by the CDC(Centers for Disease Control and Prevention)[5]. The NHANES survey combines interviews and physical examinations. All the data are from 2009 to 2018, and the research sample is aged from 20 to 79 years old. R 4.2.2, and Excel 2016 are used to analyze our data. The variables involved are age, gender, and BMI, which is conducted by weight divided by height square. Education level is divided into three categories: college graduate or above, high school or below. It was divided in this level because people from different educational levels have different kinds of pressure. Some variables were obtained from the questionnaire: Sleeping status, Smoking status. As these two are related with daily performance in both work and daily life. Sleeping status was measured by asking the behind questions in the questionnaire: Ever told a doctor had trouble sleeping? Smoking status was asked by Have you smoked at least 100 cigarettes in your entire life? Arthritis and Coronary heart disease are measured by doctors' diagnoses. And some disease information, in our project, we only explore two diseases, Arthritis, and coronary heart disease. The mathematical method we used is the Chi-square test, t-test, and adjusted logistic regression analysis.

3. Results

In logistic regression, three models are set up: Model 1, Adjusted for gender and age; Model 2, Adjusted for all the collected factors; Model 3, Adjusted for age, gender, Education level, Marital status, Smoking status, BMI, and Arthritis. And Depression was measured using the Patient Health Questionnaire (PHQ-9), a nine-item screening instrument that asked questions about the frequency of symptoms of depression over the past 2 weeks. Response categories for the nine-item instrument "not at all," "several days," "more than half the days" and "nearly every day" were given a point ranging from 0 to 3. According to the answer to each question, we can calculate the whole score of this questionnaire. A total score $\geqslant 10$ was considered to be clinically relevant depression (CRD).

Table 1. Baseline table.

Characteristic	Having sleeping trouble					
	level	overall	No	Yes	p-value	test
a		24804	18159	6638		
age(mean(SD))		49.8(17.7)	48.6(18.0)	53.1(16.3)	<0.01	t-test
education(N)	collage	7592(30.6)	5341(29.4)	2250(33.9)	<0.01	Chi-squared test
	graduate or above	5972(24.1)	4521(24.9)	1448(21.8)		
	High school or below	11218(45.3)	8278(45.6)	2938(44.3)		
Marital(h)	77	15(0.1)	11(0.1)	4(0.1)	<0.01	Chi-squared test
	99	1(0.0)	0(0.0)	1(0.0)		
	coupled	14596(58.8)	10993(60.5)	3601(54.2)		
	Single or separated	10192(41.1)	7155(39.4)	3032(45.7)		
gender(N)	Male	12194(49.2)	9416(51.9)	2775(41.8)	<0.01	Chi-squared test
	Female	12610(50.8)	8743(48.1)	3863(58.2)		

DOI: 10.54254/2753-8818/21/20230893

Table 1. (continued).

Medium score(mean(SD))		3.4(4.7)	2.5(3.9)	5.7(5.7)	<0.01	t-test
Smoking status(N)	No	13870(55.9)	10775(59.4)	3090(46.6)	<0.01	Chi-squared test
	Yes	10920(44.1)	7374(40.6)	3545(53.4)		
BMI(mean(SD))		29.4(7.1)	28.9(6.7)	30.8(8.0)	<0.01	t-test
Arthritis(N)	NA	9113(36.8)	6834(37.7)	2278(34.4)	<0.01	Chi-squared test
	No	11275(45.5)	8930(49.2)	2343(35.4)		
	Yes	4382(17.7)	2374(13.1)	2004(30.2)		
Coronary heart disease(N)	NA	9113(36.8)	6834(37.7)	2278(34.4)	<0.01	Chi-squared test
	No	14971(60.5)	10923(60.3)	4043(61.1)		
	Yes	668(2.7)	369(2.0)	298(4.5)		
Race(N)	Mexican American	2636(13.5)	2097(14.8)	539(10.2)	<0.01	Chi-squared test
	Hispanic	2003(10.3)	1500(10.6)	503(9.5)		
	White	7397(37.9)	4899(34.5)	2497(47.1)		
	Black	4432(22.7)	3269(23.0)	1160(21.9)		
	Asian	2296(11.8)	1956(13.8)	339(6.4)		
	Other	730(3.7)	470(3.3)	259(4.9)		

This is the baseline table. It is found that of all Of the 24804 subjects (49.2% males and 50.8% females, mean (SD) age 49.8 [17.7] years), the p-value of different Age, Education level, Marital status, gender, Smoking status, BMI, Race, and disease groups are less than 0.05, which shows that they are significantly related to the Sleeping disorder.

3 models are designed for logistic regression. The result is shown in Tables 2-4.

Table 2. The result of logistic regression of Model 1.

Var	OR	p-value	OR1	OR2	OR mean
Trouble sleeping	4.54(4.16~4.97)	<0.0001	4.156367965	4.96814356	4.543327991
Age	1(0.99~1)	0.003	0.993613365	0.998728191	0.996169243
Gender	1.5(1.38~1.65)	<0.0001	1.375374458	1.646930581	1.504687968

Table 3. The result of logistic regression of Model 2.

Var	OR	p-value	OR1	OR2	OR mean
Trouble sleeping	4.24(3.75~4.8)	<0.001	3.753297522	4.795376581	4.240895346
Age	0.99(0.99~1)	<0.001	0.987443405	0.995181901	0.99131623
Educational level	0.74(0.7~0.78)	<0.001	0.70256007	0.775195789	0.738015144
Marital status	1.04(1.02~1.06)	<0.001	1.020754063	1.057893579	1.039468537
Gender	1.57(1.39~1.78)	<0.001	1.393657078	1.781328587	1.574997958
Smoking status	0.61(0.54~0.68)	<0.001	0.536213553	0.682885507	0.605299766
Race	1(0.96~1.04)	0.868	0.957214624	1.037177098	0.996601535
BMI	1.02(1.01~1.02)	<0.001	1.009172704	1.024012526	1.016602483
Coronary heart disease	0.98(0.86~1.1)	0.741	0.859821311	1.096823652	0.979865967
Arthritis	0.65(0.57~0.75)	<0.001	0.571773244	0.745065927	0.653733091

DOI: 10.54254/2753-8818/21/20230893

Table 4. The result of logistic regression of Model 3.

Var	OR	p-value	OR1	OR2
Trouble sleeping	4.23(3.75~4.77)	<0.001	3.753750984	4.773417895
Age	0.99(0.99~0.99)	<0.001	0.987069512	0.99465139
Education	0.74(0.7~0.77)	<0.001	0.701860059	0.770532637
Marital	1.04(1.02~1.06)	<0.001	1.022175482	1.059048497
Gender	1.58(1.4~1.78)	<0.001	1.397945478	1.778391305
Smoking status	0.61(0.54~0.69)	<0.001	0.541177175	0.685988057
BMI	1.02(1.01~1.02)	<0.001	1.009262954	1.023840267
Arthritis	0.65(0.57~0.74)	<0.001	0.572713798	0.742980384

Model 1:is adjusted for age and gender, (OR : 4.54 (4.16~4.97). Model 2 is adjusted for age, gender, Education level, Marital status, Smoking status, Race, BMI, and two diseases（ACD and Arthritis) (OR : 4.24 (3.75~4.8)). Model 3 removed variables in model 2 where p-value is greater than 0.05, Adjusted for age, gender, Education level, Marital status, Smoking status, BMI, and Arthritis(OR : 4.23 (3.75~4.77)).

Since in the background information, it is learned that depression is significantly related to gender and age differences, the first model only takes gender and age as covariates to examine the relationship between depression and sleeping trouble. The OR value (confidence intervals) of sleeping trouble is 4.54 (4.16-4.97), age is 1 (0.99-1), gender is 1.5 (1.38-1.65), and all the p-values are less than 0.05. In the baseline table, it is learned that all variables had significant differences in the presence or absence of sleep trouble, so all variables are tried to set as covariates and consider their relationship with depression. In the second model, the OR value (confidence interval) of trouble sleeping and depression has dropped to 4.24 (3.75-4.8). Nearly all the variables' p-value is less than 0.001, except race and Coronary heart disease. Lastly, due to the variables' p-value being larger than 0.05, these two variables are removed, not setting them as covariates, and again examine the relationship between the remaining eight variables and the depression. The OR value (confidence interval) of trouble sleeping has dropped to 4.23 (3.75-4.77).

Then R's forest plot package is used to draw a forest plot. The numbers show the OR value and its confidence interval. And the most right is the p-value. As shown in fig4, in the age- and gender-adjusted model (model 1), sleeping trouble (OR 4.54, 95% CI: 4.16-4.97) was substantially related to CRD. In model 2(OR 4.24, 95% CI: 3.75-4.8) and model 3(OR 4.23, 95% CI: 3.75-4.77), the sleeping trouble and CRD remained significant after adjusting for potential confounding factors.

DOI: 10.54254/2753-8818/21/20230893

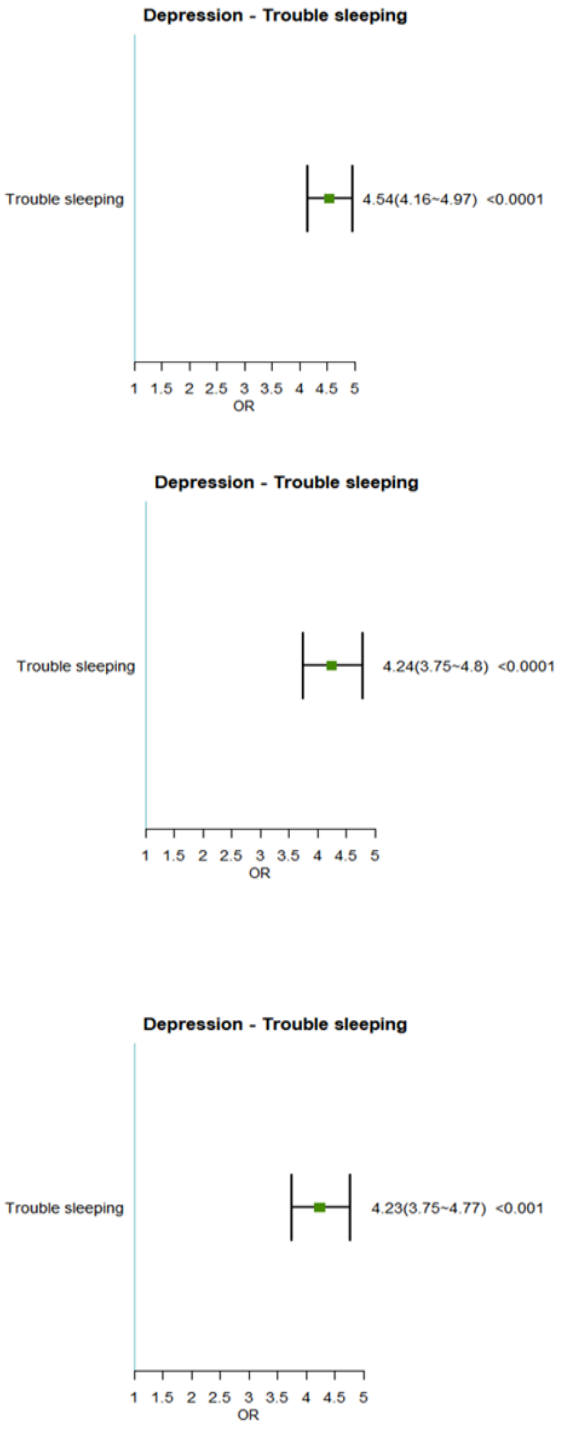

Figure 1. Forest plot.

4. Discussion

In the research data is searched from enhances, maybe more data can be found from different resources, and we can also minimize the scale of the sample. 49000 data are collected after the detection of NA, there are only 25000. More data from different resources can be found and more samples can be collected. In the aspect of the sample, a more specific sample range can be decided, for example, all the students

DOI: 10.54254/2753-8818/21/20230893

in a university. Dinis et al did their research on depression and the quality of sleep in college students [6], a suitable group can also be found to improve the research.

We drew the baseline table of our data and use logistics to analyze it. To improve our analysis, more advanced methods can be used, for example, hypothesis testing and a better-adjusted logistic model. More disease information and better measurement of sleeping trouble/sleeping status will make our study stricter. Other studies have shown that depression is associated with many other diseases, more data can be collected from other diseases besides the two diseases we analyzed. Also, more specific criteria for sleeping trouble can be found, the question mentioned in NHANES is very subjective, if an objective one can be found then our results will be more referential. Baglioni et al did their research on the relationship between insomnia and depression, and they collected their data from the previous study done by others [7]. In that case, more diseases that are suitable for research can be found and researched and data from previous studies can be found. There is a study which did research on the association between depression and neurological changes. It concluded that the depression influence the neuro to be less active and get down the quality of sleep [8].

5. Conclusion

The self-reported trouble sleeping was significantly related to CRD (OR: 4.23, 95% CI: 3.75-4.77). There are a lot of factors related to CRD, in the study, age, gender, education level, marital status, smoking status, obesity degree and also some diseases are confirmed to have an association with CRD. However, of all the factors that are analyzed, Sleeping disorder has the biggest OR value, which means it is most related to CRD. The more sleeping disorder the patients have, the more CRD patients have. Age, martial status and gender have relationship with CRD but the relationship is related to different groups in these two conditions. Educational level and smoking status have the least relationship with CRD because of the lowest OR value.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230894

Analysis on the influence of a low-carbohydrate diet and a high-protein diet on the glucose level of diabetes

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Abstract. The treatment of type 2 diabetes has been greatly aided by dietary changes. The practice of adopting a healthy diet has become increasingly popular around the world. In addition to assisting with the maintenance of appropriate blood sugar and blood pressure levels, this also helps to prevent long-term organ damage and enhances general health. In this analysis, we look at the research on high-protein and low-carbohydrate diets for diabetes patients with the goal of better glycemic control. To better understand the connection between type 2 diabetes and food, this study will examine the effects of a high-protein diet and a low-carbohydrate diet on blood sugar levels in persons with the disease.

Keywords: high-protein diet, low-carbohydrate diet, glucose level, type 2 diabetes.

1. Introduction

Type 2 diabetes is a non-communicable disease that is becoming increasingly common. Non-insulin dependent diabetes mellitus (NIDDM), also known as Type 2 diabetes, accounts for about 90% of all occurrences of diabetes. Type 2 diabetes has emerged as one of the public health crises with the fastest global expansion due to modern unhealthy lifestyle and eating habits, which are expected to double the number of victims within the next 25 years. The World Health Organization estimates that 642 million people will have diabetes by 2040, making it the most common chronic disease in the world. And it caused serious damage to total body health as limiting results, such as heart disease, peripheral vascular disease, and retinal aberrations that could lead to blindness, difficulties that carry heavy financial and medical costs. Diet plays a significant role in defining human health, and making dietary adjustments can aid in the control of diabetes. Glycemic control and nutritional health can both benefit from a better understanding of the effects of different diets on diabetes patients.

To begin, the dietary factors that can affect one's risk of developing diabetes are the primary emphasis of this review. Substantial study on the link between health and food indicates that nutrition is an important element in diabetes control, making dietary management critical for the body. Most of the previous studies have either emphasized the importance of high protein or low carbs. Even while low-carbohydrate, high-protein diets are gaining popularity, the data regarding their efficacy in the management of diabetes is mixed. Taking their impact on blood sugar levels into account can help provide more solid proof for or against their usage in diabetes management.

Second, the effectiveness of a high-protein diet and a low-carbohydrate diet in managing blood sugar levels in persons with type 2 diabetes is compared and contrasted in this systematic study. The goal is to

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DOI: 10.54254/2753-8818/21/20230894

give patients more information about how different diets affect diabetes care so that they can create programs that work for them based on factors like age, weight, and profession. By focusing on the benefits of limiting consumption of complex carbs and dietary protein, this study hopes to provide light on the potential advantages of these diets for persons with type 2 diabetes.

2. Diabetes

Diabetes mellitus is a metabolic illness marked by high blood glucose levels and glycosuria, and is caused by damage to pancreatic beta cells and insulin resistance. Glucose or sugar is generally produced by the body after digestion of food and is used for energy. However, those with diabetes tend to have higher blood glucose levels because they either don't make enough insulin or are unable to properly utilize the insulin they do create. Protein and lipid metabolism both undergo changes in later phases. Diabetes risk increases with age, obesity, poor diet, lack of exercise, higher socioeconomic status, higher blood pressure, and higher levels of psychological stress. Complications from high blood sugar include blindness, kidney failure, nerve damage, and an increased chance of developing cardiovascular disease and high blood pressure. The two most frequent forms of diabetes are type 1 and type 2. Blurred vision, a painful throat, increased thirst, frequent urination (especially at night), and blurred vision are all symptoms of untreated diabetes. In order to properly treat diabetes, blood glucose levels must be maintained through a combination of dietary and physical activity restrictions, medication, and close monitoring.

2.1. Type-2 diabetes

Approximately 90–95% of all instances of diabetes are attributable to type-2 diabetes, also known as non-insulin-dependent (NIDD) diabetes. This form of diabetes often affects adults, and it develops slowly over time. It might take up to ten years for someone to be diagnosed with high blood glucose levels because the condition often has no symptoms until complications arise. Both insufficient insulin synthesis by the pancreas and insulin resistance in the body's cells contribute to the impaired glucose uptake seen in type 2 diabetes. There may be an abundance of insulin in the body, but if the cells are immune to its effects, glucose will continue to build up in the blood. The symptoms of being overweight, being middle-aged or older, and having a family history of diabetes are all present in a sizable percentage of patients with type-2 diabetes. Although it typically manifests in those 40 and older, it can strike younger people, particularly those of South Asian and African-Caribbean descent. Renal failure, blindness, heart disease, and stroke are some of the complications of diabetes that can occur if the condition is not addressed. Treatment options include alterations to the patient's diet and exercise routine, as well as medication and insulin injections. The onset of Type-2 diabetes may be postponed or avoided entirely with the help of lifestyle modifications including increasing physical activity and keeping the weight where it should be. With the correct diagnostics, medical attention, and dietary adjustments, type-2 diabetes can be effectively controlled.

2.2. Effect of low carbohydrates on type 2 diabetes

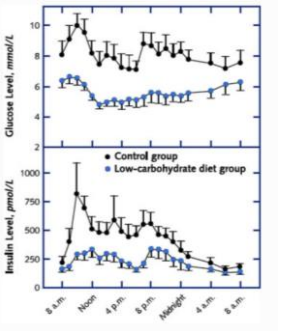

Figure 1. The concentration level of glucose and Insulin after implementing low carbohydrate diet after 24 hours [1].

DOI: 10.54254/2753-8818/21/20230894

For better blood sugar control and overall health, many people have turned to low-carbohydrate diets, in which they consume fewer carbs such as sugars and starches. The amount of carbohydrates eaten is cut back from what would be considered a normal or high-carb diet. In the first study, the low-carbohydrate diet group had lower post-meal and fasting glucose levels. In this study, eight individuals with untreated type-2 diabetes were given a LoBAG diet, a high-protein, low-carbohydrate diet. Weight-maintaining and non-ketogenic, the diet's carbohydrate:protein:fat ratio was 20:30:50. 55:15:30 was the control diet. A 5-week washout was used in the randomized crossover experiment.

The integrated glucose concentration over the course of 24 hours dropped dramatically as a result (Fig.1). Thus, a low-carbohydrate diet lowered both post-meal and fasting glucose levels, as well as 24-hour and 5-week integrated glucose levels and the percentage of glycohemoglobin concentration.

A 24-week research comparing a low-glycemic, reduced-calorie diet to a low-carbohydrate diet for patients with obesity and type-2 diabetes found the former to be superior in terms of glycemic management [2]. Group meetings, nutritional supplements, and exercise advice were all included in the two diet plans that were randomly assigned to the 84 study participants. The outcomes demonstrated reductions in weight, glucose, and insulin levels after both therapies. Hemoglobin A1c, body weight, and high-density lipoprotein cholesterol all improved in both groups, although the low-carbohydrate group reduced or eliminated their diabetic medication more often. Low-carbohydrate therapy and other lifestyle adjustments were found to improve and in some cases reverse diabetes in patients with type 2 in the study.

2.3. Effects of high protein on type2 diabetes

Previous studies shown that after 5 weeks, glycohemoglobin levels decreased significantly (from 8.1% to 7.3%) when carbohydrate intake was reduced and protein intake was increased from 15% to 30% of total food energy [4]. Despite lower post-meal glucose levels, the fasting glucose concentration did not change.

This follow-up study aimed to further reduce the carbohydrate content of the diet while maintaining the 30 percent protein intake found in the first. In example, the proportion of glycogen to total meal energy went down from 40% to 20%. The control group in all studies followed a diet comparable to that suggested for reducing coronary heart disease risk [5]. This 24-week trial compared a low-carbohydrate diet to a low-glycemic, reduced-calorie diet on glycemic management in obese and type 2 diabetic patients. Group meetings, nutritional supplements, and exercise advice were all included in the two diet plans that were randomly assigned to the 84 study participants. The outcomes demonstrated reductions in weight, glucose, and insulin levels after both therapies. The low-carbohydrate group, however, saw bigger reductions or eliminations in the use of diabetes medications, as well as greater improvements in hemoglobin A1c, body weight, and high density lipoprotein cholesterol. The results of the study show that people with type 2 diabetes can improve their condition and even reverse it with the help of low-carbohydrate therapies as part of a healthier lifestyle.

Another study confirmed the same benefit from adopting a high-protein diet [7]. This study examined the metabolic health of untreated type 2 diabetics on a high-protein diet versus a control diet. The macronutrient breakdown of the high-protein diet was 30:40:30, while that of the control diet was 15:55:30. The patients stayed the same weight throughout the research despite following either diet for a total of 5 weeks. In comparison to the control diet, the high-protein diet was found to improve glucose management by lowering the mean 24-hour integrated glucose area response by 40%. Glycated hemoglobin levels were reduced by 0.8% on the high-protein diet compared to 0.3% on the control diet. High-protein diet participants also showed a considerably faster rate of change over time compared to the control diet participants [8]. The high-protein diet lowered fasting triacylglycerol relative to the control diet. Insulin, C-peptide, and free fatty acid concentrations were similar between diets [9].

Based on the results of these two studies, it appears that a high-protein diet may improve glucose management in persons with type 2 diabetes when compared to a control diet. To determine the entire amount of the reaction, any potential negative effects, and the diet's long-term acceptability, however, more research is needed over longer time periods.

DOI: 10.54254/2753-8818/21/20230894

3. Suggestions

Longer intervention periods (say, 12 or 24 weeks) might help researchers learn more about the long-term impact of a low-carbohydrate diet on glucose management for those with type-2 diabetes. Researchers would be able to investigate the potential for long-term consequences of any benefits discovered if the intervention was larger in scope. The statistical power and generalizability of the study could also be improved by increasing the size of the sample. A larger sample size would also permit a more in-depth analysis of the potential variations in how the low-carb diet affects glucose control in various subgroups of people with type 2 diabetes, such as those with different degrees of insulin resistance or different HbA1c readings at the outset of the study. More data on critical outcomes like weight loss, blood pressure reduction, and lipid profiles may be able to paint a fuller picture of the impact of a low-carbohydrate diet on the health of people with type-2 diabetes. More definitive results on the effect of the low-carbohydrate diet on glucose control and other health outcomes in patients with type-2 diabetes require a larger, longer-term experiment. More information on these results might help doctors better care for their type-2 diabetic patients. This is for the best interests of the patients.

4. Conclusion

Effective lifestyle changes in preventing type-2 diabetes include adopting a balanced food pattern and engaging in regular physical activity. Therefore, it is essential to place emphasis on encouraging people, especially those at high risk, to adopt healthier lifestyle choices such as eating a balanced diet. The results of this research add to the mounting evidence supporting the advocacy of a high-protein, low-carbohydrate diet. Due to the lack of true experimental settings for exploring the topic in the real world, the work has certain limitations; for example, the variables (study length, protein/carbohydrate intake) leading to the result are not fully identical. As a result, this review can only provide a broad assessment of the papers included in it.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230897

Pathway analysis of beneficial bacteria in suppression of plant immunity

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Abstract. Plants, much like animals, possess immune mechanisms that help protect them against harmful microorganisms and pathogens. Research studies have provided evidence that various detrimental microorganisms can target the immune system of plant roots in the soil, leading to plant diseases. At the same time, recent studies have shown that beneficial microorganisms can also suppress the plant's immune system and form a mutually beneficial relationship with the plant, promoting the colonization of beneficial bacteria and helping the plant to defend itself against other harmful microorganisms. This paper provides a summary of the concept that beneficial bacteria hinder root immunity by inhibiting various downstream reactions within the plant's immune system, based on the understanding of plant root immune mechanisms. The authors also highlight the existing gaps in research on non-invasive microbe-plant interactions and raise questions regarding the intricate interplay between immunity and symbiotic signaling.

Keywords: beneficial bacteria, pathways, plant immunity, immune mechanisms.

1. Introduction

It is widely recognized that the soil harbors a rich diversity of bacteria [1]. Plants, thriving within the expansive natural petri dish of soil, are subject to intricate and diverse interactions between their root systems and a multitude of microorganisms. These interactions play a significant role in modifying the physiological state of plants and reshaping the microbiota residing in the inter-root region [2]. During such interactions, harmful microorganisms, such as pathogens, can attack the immune system and prevent healthy plant growth by controlling the metabolism of plants, even killing the plant [3]; simultaneously, beneficial microorganisms have been recognized for their ability to prevent plant diseases and support vital physiological functions by enhancing microbial activity [4]. However, a growing number of studies have shown that beneficial microbes can also suppress the plant's immune system and establish a mutually beneficial relationship with the host [5] This symbiotic relationship not only enables the beneficial bacteria to thrive within the plant's root zone but also empowers the plant to defend itself against detrimental bacteria [6]. Moreover, the beneficial bacteria can assist the plant in self-protection through the secretion of proteins that serve as elicitors and effectors [7]. This article aims to provide a comprehensive review by organizing the existing literature from two perspectives: (1) the pathway of the plant root immune mechanism, and (2) the inhibitory and regulatory effects of probiotics on the plant root immune mechanism.

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DOI: 10.54254/2753-8818/21/20230897

2. Immune mechanisms in plant roots

The innate immune response serves as the mechanism through which plants defend themselves against pathogen attacks. This immune response comprises two distinct layers, each playing a crucial role in protecting the plant from pathogens [8]. The initiation of these two layers of the innate immune system requires a cell surface-localized pattern recognition receptor (PRR) and an intracellular nucleotide-binding domain leucine-rich repeat sequence receptor (NLR), respectively, and leads to pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively [9]. Despite their differences, these two layers of the innate immune system share numerous downstream reactions and engage in complex interactions [9]. Common downstream reactions in the plant innate immune system include the activation of the mitogen-activated protein kinase (MAPK) cascade [10], the generation of reactive oxygen species (ROS) burst, the influx of ions (such as calcium flux), transcriptional reprogramming, and the enhanced synthesis of plant defense hormones [11]. These downstream reactions collectively contribute to the establishment of a robust immune mechanism in plants [9]. Importantly, these immune mechanisms are not limited to specific plant parts, but are applied throughout the entire plant. Specifically, the root system, which is submerged in the soil, is also capable of initiating a pattern recognition receptor (PRR)-mediated immune response [12]. The underlying principle is that pattern recognition receptors (PRRs) in plant roots have the ability to detect the presence of abundant microorganisms in the soil by recognizing specific microbial-associated molecular patterns (MAMPs). In response to this recognition, the PRRs can trigger an immune response or activate defense genes, leading to various outcomes such as the induction of cellular damage at the root site and the initiation of a localized immune response [1, 12, 13].*Immunosuppression of plants by beneficial bacteria*

After reviewing immune mechanisms in the plant root system, it is noteworthy that beneficial microorganisms can elicit responses in plants that closely resemble those triggered by pathogens [13, 14]. In other words, beneficial bacteria possess microbial-associated molecular pattern (MAMP) analogs that allow them to interact with and activate pattern recognition receptors (PRRs) in plant roots, leading to the stimulation of immune signals [13, 14]. Moreover, in the later stages of interaction between beneficial bacteria and plant roots, symbiotic bacteria can block plant defence mechanisms and thus remain in the plant root interiors [14].

It is known from the immune pathways of plants that diverse downstream responses are essential to ensure the immune mechanisms of plants [9]. Therefore, it is reasonable to speculate that beneficial bacteria have the capability to suppress plant immunity by inhibiting or modulating these downstream reactions.

The first avenue of exploration focuses on the MAPK cascade. Previous studies have shed light on the inhibitory effects of nodule exoprotein L (NopL), a type III effector produced by the symbiotic bacterium Rhizobium, on plant innate immunity [15]. Notably, NopL is regarded as a crucial virulence factor in plant-pathogen interactions [15]. Experimental evidence from tobacco (Nicotiana tabacum) reveals that the expression of NopL can impede the full induction of pathogen-associated (PR) defense proteins, thus effectively suppressing the plant's innate immune response [16]. In a more targeted experiment, the influence of NopL on the MAPK cascade was investigated using Agrobacterium-mediated transient transformation of tobacco. The results demonstrated that co-expression of NopL and SIPK (salicylate-inducible protein kinase) in tobacco led to a noticeable reduction in cell necrosis within the leaf region, which is typically associated with the overexpression of the MAPK SIPK gene [17]. This observation strongly suggests that the co-expression of NopL and SIPK disrupts MAPK signaling pathways. Moreover, additional investigations revealed that the presence of SIPK DD (a mutant form of SIPK) directly triggers cell death [17]. These experimental results suggest that NopL from beneficial microorganisms has a repressive effect on the function of MAPK proteins and their downstream transcription factors [17].

The second concept revolves around the regulation of reactive oxygen species (ROS) bursts, as exemplified by the effector PIIN_08944 derived from the beneficial microorganism Piriformospora indica (P. indica) [18]. P. indica is a tamerobacterium that colonizes the roots of various plants as a beneficial bacterium [19]. To investigate the impact of PIIN_08944 on ROS burst in plants, experimental

DOI: 10.54254/2753-8818/21/20230897

procedures involved the use of quantitative polymerase chain reaction (qPCR) to confirm the expression of PIIN_08944 in plant roots. Subsequently, transgenic barley plants fused to PIIN_08944 and GFP (HvPIIN_08944OE plants) with peptide flg22 and titin to measure the production of ROS in the plant. It was obtained that overexpression of PIIN_08944 significantly reduced the conclusion that flg22 and tylosin-induced ROS burst in HvPIIN_08944OE [18].

In addition, the modulation of hormonal signaling is recognized as an essential mechanism to suppress plant immunity. To explore the regulation of plant immunity through hormonal signaling, researchers conducted an investigation focusing on the promoters of flg22-triggered plant defense. Specifically, they examined the response of CYP71A12, MYB51, and WRKY11 promoters in plants with root treatments of the beneficial bacterial rhizobacterium FB17[20]. The results indicated a significant inhibition of these promoters in plants treated with FB17, suggesting that FB17 suppresses the flg22-induced root defense responses that are typically activated by microbial-associated molecular patterns (MAMPs) [20].

2.2. Beneficial bacteria colonize the root system

According to the pathway mentioned above of immunosuppression of plants by beneficial bacteria, it is not difficult to find that some beneficial bacteria promote plant root colonization in this process. For example, in the P. indica effector PIIN_08944 mentioned above, experiments have concluded that PIIN_08944 promotes fungal colonization of barley roots by using PIIN_08944-deficient mutants grown on medium and comparing the colonization of plant roots in the standard group [18]. Moreover, beneficial rhizobia FB17 performed rhizobia inoculation of Arabidopsis wild-type plant leaves by spraying with MAMPS, stimulating the colonization and quantification of FB17, indicating that plants can recruit beneficial rhizobia underground [20].

3. Discussion

The aforementioned examples provide compelling evidence for the immunomodulatory strategies employed by beneficial microbes during their invasion of host roots. These studies highlight the effectiveness of suppressing plant root immunity through the inhibition of downstream signals in the immune machinery. The inhibition of MAPK, ROS burst, and hormone signalling molecules are exemplified for the downstream responses of plant immunity. It is shown that colonization of plant roots by beneficial bacteria can be achieved in these pathways, thus recruiting other fungi or microorganisms to protect the plant better. At the same time, existing studies have shown that non-invasive beneficial microbes can also modulate plant immunity. For example, beneficial Pseudomonas can suppress multiple downstream flg22-dependent root immunity by acidifying the environment [21], and another example is the use of high-throughput transposon sequencing (Tn-Seq) to search for genes in Pseudomonas _ WCS365 that prevent the formation of dense biofilms in roots to evade plant defence mechanisms [22]. Furthermore, these studies also affirmed that these pathways could promote the colonization ability of microorganisms [21,22]. Nevertheless, in the context of the diverse and intricate immune interactions between plant roots and the myriad microorganisms present in the soil, it is imperative to explore the potential interplay between these different pathways. Specifically, understanding whether invasive beneficial bacteria and non-invasive microorganisms exhibit antagonistic or synergistic effects on each other represents a crucial and intriguing area for future investigation.

4. Conclusions

In conclusion, this study has provided insights into the diverse mechanisms through which beneficial bacteria can suppress plant immunity. By elucidating these mechanisms, the paper highlights the ability of plants with suppressed immune systems to still defend themselves against harmful microbes. This symbiotic relationship between beneficial bacteria and plants allows for the colonization of plant roots and provides enhanced protection. While this article offers a comprehensive literature review on the diverse mechanisms by which beneficial bacteria influence plant immunity, its limitations lie in the

absence of empirical validation. It is crucial for future research to prioritize experimental studies and utilize alternative methodologies to gather firsthand data, allowing for a more in-depth exploration of this subject. By incorporating empirical evidence, researchers can delve further into the specific interactions and molecular mechanisms that underlie the suppression of plant immunity by beneficial bacteria. This will contribute to a comprehensive understanding of complex plant-microbe interactions and establish a solid foundation for the development of practical applications in the fields of agriculture and environmental management.

Acknowledgment

I would like to express my sincere gratitude to my mentors for their invaluable guidance and unwavering support throughout this research journey. Their expertise and insights have been instrumental in shaping this study. I am also grateful to my colleagues, friends, and family for their constant encouragement and assistance, which has been a source of motivation for me. Their belief in me has made this research possible.

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230899

Limitations of CAR-T therapy and possible directions of improvement

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Abstract. Chimeric antigen receptor T-cell (CAR-T) therapy is a new biological immunotherapy approach. This paper is an overview of the limitations of CAR-T therapy and possible directions of improvement. This paper starts with the mechanism of CAR-T, briefly describes the pathogenesis, and then provides a detailed description of the three types of CAR-T toxicity and their mechanisms, including cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and CAR-T-associated encephaly syndrome (CRES). In the end, the paper proposes several possible improvements to improve the current situation, summarizes the corresponding improvements, and looks forward to the future.

Keywords: CAR-T, toxicity, CRS, ICANS, CRES.

1. Introduction

The goal of CAR-T therapy is to isolate and remove T lymphocytes from the patient's body, then use genetic engineering technology to transform, process, and culture them. Then, T cells are activated and given the CAR gene, which allows T cells to specifically recognize tumor cells, and release a significant number of effector factors by immunological activity. Finally, the cells were injected back into body to alter proteins at the site of the tumor to eliminate or reduce the destructive power of cancer cells. It is mainly used for refractory malignant hematologic diseases, such as leukocyte, lymphoma, multiple myeloma and so on. CAR-T therapy can treat cancer accurately, quickly and efficiently. However, this method also has certain adverse reactions, patients may have high fever, chills, nausea, muscle pain, general edema, cardiac insufficiency, breathing difficulties, kidney function damage. Due to the incidence and resulting burden of CRS, CRES, and ICANS in therapy, the use of CAR-T therapy is now restricted to some extent [1].

In many studies now, researchers have suggested ways to deal with these side effects. This paper will summarize the mechanism of CAR-T therapy, three toxicities and possible ideas for improvement, hoping to provide a guide for the future development.

2. Mechanism

CAR-T therapy is the development of antigen-binding T cell receptors containing immunoglobulins through genetic cloning. Genetic material containing specific CAR domains and T cell activation signals

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DOI: 10.54254/2753-8818/21/20230899

has been introduced into T cells. As a result, they bind directly to specific antigens on the surface of tumor cells and activate them [2].

After recognizing tumor antigens, CAR activates the immune pathway of the working cells, and the expression of related genes is up regulated. Granulocyte, perforin, and other substances are secreted to directly mediate tumor cell lysis. Meanwhile, inflammatory factors, such as GM-CSF, is also secreted.

Several key points are involved in the application of CAR-T therapy: antigen binding site, binding, transmembrane domain and signal transduction domain. These are also important areas of concern for improving CAR-T therapy.

3. Toxicity

3.1. CRS

The most frequent immediate side effect of CAR-T treatment is CRS. CAR-T cells' activation following tumor detection in vivo causes the production of cytokines, which in turn causes systemic inflammatory reactions, or CRS. The pathophysiology of CRS is influenced by inflammatory cytokines, which are released by bystander immune cells such macrophages after being activated by CAR-T cells. In more severe cases, CRS can result in numerous organ malfunction. It typically manifests as systemic symptoms such as fever, myalgia, chills, lethargy, and appetite loss. CRS are entirely reversible, though, if handled correctly [3].

In the five stages, "CRS" has the following pathological changes: In the first and second stages, many malignant cells spread to the tumor source, CAR-T cells recognize and proliferate, produce cytokines to trigger the occurrence of CRS, and kill the tumor cell. In phase 3, cytokinesis actively enters the peripheral blood, and systemic inflammation occurs concurrently. This is because the CAR-T cell population in the peripheral blood increases, which can cause complications such as hypoxia, hypotension, and organ damage. This is an imbalance of osmotic pressure caused by damage to multiple organs or tissues, as well as holes in blood vessels with leakage and endothelial damage. In the fourth stage, peripheral blood enters the cerebrospinal fluid (CSF) and central nervous system (CNS) by allowing cytokine diffusion, CAR-T cells, endogenous T cells, and peripheral activated monocytes, including the destruction of the blood-brain barrier (BBB) [4]. In turn, it leads to the remission of symptoms in the fifth stage: the decrease of cytokines, the weakening of systemic inflammation, the gradual end of CRS/ICANS symptoms, and the generation of long-standing memory T cells. It has been demonstrated that serum IL-6 levels are correlated with the severity of CRS, and that tolumab, an anti-IL-6 receptor antibody, blocks IL-6 to reverse CRS. It has also been demonstrated that other cytokines and chemokines, including IL-8, IL-10, IL-15, IFN-, and MCP-1, are related to severe CRS. Patients with CRS and mice models have both shown elevated levels of these key cytokines. Corticosteroids are employed as a result because they produce global immunosuppression, decrease the production of several cytokines and chemokines, and directly impact CAR-T cell proliferation and function, if blocking IL-6 alone is insufficient to manage CRS. For the clinical management of CRS, tocilizumab and other core cytokine blocking inhibitors have also been approved as treatment options. CRS can be diagnosed by tracing the genesis of its main cytokines.

At now, it makes sense for researchers to concentrate on IL-6 production to understand the mechanism of CRS [4]. It has been demonstrated that IL-6 is a pleiotropic cytokine released under a variety of circumstances, including stress, infection, and tissue damage. In addition to immune cells, fibroblasts, mesenchymal cells, and vascular endothelial cells also release IL-6.

Uncertainty surrounds the pathophysiological mechanism of CRS. The primary source of core cytokines in CRS may be macrophages. The combination of modified T cells with their target cells can cause on-target effects, which can then attract and activate bystander immune or non-immune cells. Myeloid macrophages have been implicated in the pathophysiology of CRS in an increasing number of studies.

Macrophages were the primary source of Nitric oxide synthase (iNOS), a kind of inflammatory cytokine. Vasodilation and hypotension, which are serious life-threatening clinical symptoms during

DOI: 10.54254/2753-8818/21/20230899

CRS brought on by the infusion of CAR-T, are produced by abnormal NO generation. Treatment with the iNOS inhibitor L-NIL or 1400 W in Sadelain's trial both increased survival and decreased toxicity in the case of severe CRS. Yet, there is also evidence that iNOS are engaged in additional CRS processes. In plasma cells, George et al. discovered that IL-6 promoted Nos 2 transcription. Furthermore, IL-1 can promote iNOS expression and synthesis. IL-6 and IL-1 strongly induce CRS, and iNOS is thought to be involved in this process [5].

3.2. ICANS

ICANS can happen while having CRS or, more frequently, after it has passed. In more severe cases, it can develop to lower levels of awareness, coma, seizures, motor weakness, and cerebral edema. It often manifests as toxic encephalopathy with difficulties speaking, aphasia, and bewilderment. Similar to CRS, the degree of neurotoxicity was linked with cytokine, chemokine, and CAR-T cell amplification [6].

The neurotoxicity may result from endothelial cell activation and BBB disruption. 10 Other findings point to the CNS's involvement with myeloid cell activation. The relevance of IL-1 in the pathophysiology of the neurotoxicity has recently been shown in two mice models, and both toxicities may be reversed by inhibiting IL-1 using the IL-1 receptor antagonist analbinin. ICANS handles low-grade toxicity mostly with supportive treatment because to a poor knowledge of pathogenesis, although corticosteroids are frequently utilized to treat more severe toxicity. ICANS has a self-limiting course and is totally reversible in the majority of patients, just like CRS.

3.3. CRES

Some patients with acute B-cell leukemia develop symptoms of severe neurotoxicity after treatment with CRES CAR-T cells often manifest as toxic dementia. The first symptoms are problems concentrating, speaking and writing. Other signs and symptoms include confusion, disorientation, emotional disturbances. Aphasia, drowsiness and tremors, cerebral edema is the most severe neurotoxic complications. CRES can happen simultaneously with CRS or separately, and typically occurs 4-5 days following CAR-T cell injection [5].

CRES is often accompanied by CRS, but the pathogenesis of CRES is different from that of CRS, and the mechanism of CRES is still unclear. One mechanism for the occurrence of CRES may be the activation of independent monocytes triggered by IL-1, which then produces the expression of multiple cytokines such as IL-6, which in turn activates T cells and macrophages, increasing systemic inflammation [5]. Another possible mechanism is BBB. The destruction of BBB leads to T cell migration in the brain parenchyma and leads to elevated levels of cytokines and proteins in the cerebrospinal fluid, leading to central nervous system inflammation and toxicity. And endothelial activation can aggravate systemic inflammation and BBB destruction [7]. In addition, CAR-T cells can directly damage the CNS. The detection CSF indicates that CAR-T cells can enter CNS and that patients with neurotoxicity have a considerably larger number of CAR-T cells than patients without neurotoxicity.

4. Possible improvements

4.1. Cytokine-expressing

Upregulation of IL-12 secretion has been demonstrated in research using immunocompetent and immunocompromised mice models, however toxicity has been noted in human investigations using in vitro growth of tumor-infiltrating lymphocytes. In a different strategy, Sachdeva et al. demonstrated that in response to IL-12 induction, an IL-12 inducer arms IL-12, suggesting a potential loss of NFAT promoter activity. To connect cytokine secretion with active CAR phage activity, the IL-12 gene has really been inserted under the IL-2 Ra regulator or PDCD 1. enhances plasticity and effector activity. IL-15 preserved the naive nature of central memory T cells when it was examined in vitro and in immunoreactive mice models. decreased expression of PD-1 and elevated levels of the anti-apoptotic protein Bcl-2 [8]. When IL-15 attached to the CAR-T cell membrane, similar outcomes were seen. In a solid tumor mouse model, the release of IL-7 and CCL19 by CAR-T cells improved antitumor responses

DOI: 10.54254/2753-8818/21/20230899

and raised overall survival. In mice treated with CAR-T, both these antigen-positive and antigen-negative tumors were eliminated. T cells that release CCL 19 and IL-7.

4.2. Synchronous T cell delivery

Promoting local T-cell delivery can effectively reduce damage and avoid the risk of tumor overload. Such treatments have had some success and a degree of validation in treating specific cancers and diseases. When mice are exposed to MSC, the transgenic CSU neutralization is sufficient to prevent the spread of MSC and cell blocking, which limits immune molecules inhibition, such as pv l [8].

4.3. Improvement plans specifically for CRS

Clinical signs and severity of CRS can range greatly from non-life-threatening toxicity to severe symptoms. Accurate and prompt patient management and assessment can help prevent negative consequences [9]. After a CAR-T injection, CRS symptoms often go away in two weeks. IL-6 antagonists and/or corticosteroids may be needed for therapy, or the toxicity may be self-limiting and simply need symptomatic relief. The goal of CRS therapy is to maximize the anticancer benefits of cell therapy while minimizing hazardous side effects. There is disagreement on whether patients undergoing CAR-T treatment should be hospitalized (ZUMA-1 trial, JULIET trials, and ELIANA trial).

It is important to keep in mind that these treatment facilities have substantial expertise with CAR-T therapy and have well-established outpatient hematopoietic stem cell transplantation programs, even though this shows that the therapy may be administered in carefully supervised outpatient settings. Prior to the onset of severe symptoms, individuals who are at risk for developing severe early CRS still require rigorous monitoring and hospitalization, which requires further discovery of predictive biomarkers.

4.3.1. Supportive care. Throughout all stages of CRS, including the cell infusion, patients should receive supportive care. Complete blood counts with complete and different metabolites are often included in daily monitoring [10]. The risk of volume overload and pulmonary edema necessitates continuous monitoring of fluid balance, including daily body weight. Intravenous fluids are utilized to maintain hydration. Telemetry monitoring from CAR-T cell infusion until remission of CRS should be taken into consideration due to the danger of arrhythmia, especially in patients with concomitant cardiac risk factors.

4.3.2. Normal liver function patients. Acetaminophen can be administered to individuals with normal liver function to treat fever, and cooling blankets is another option. Alternative medications include non-steroidal anti-inflammatory medicines (NSAIDs); nevertheless, caution is needed while assessing thrombocytopenia. NSAIDs can also result in bleeding, gastritis, and renal failure. Numerous of these patients are neutropenic and have had lymphocyte clearance, therefore it is crucial to check for infections using chest x-rays, blood and urine cultures, and lymphocyte clearance. Furthermore, it is necessary to start taking broad-spectrum antibiotics.

5. Conclusion

Due to the incidence and resulting burden of CRS, CRES, and ICANS in therapy, the general use of CAR-T therapy is now restricted to some extent. The creation of efficient tailored medicines that lessen toxicity without sacrificing anticancer effectiveness will be made easier with a thorough understanding of the pathophysiology of these toxicities. New CAR architectures have been developed to enhance tumor antigen identification and efficient T-cell signaling while reducing the danger of causing CRS and ICANS. Finding a reliable and effective way to avoid these problems in the future will be one of the key components of CAR-T therapy's success.

DOI: 10.54254/2753-8818/21/20230899

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230904

Current situation and future of gene therapy for rare diseases

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Abstract. Rare genetic disorders are rare diseases that have a very low incidence, most of the patients are children, and most of them are caused by genetic defects. Many of these diseases are serious chronic genetic diseases with a small number of patients, low market demand, and high cost of drug research and development, so there is a lack of effective treatment methods, which often threaten life. Gene therapy is an emerging therapeutic approach that uses vectors to introduce genetic material into target cells to treat or prevent rare diseases by correcting or supplementing defective genes. This paper reviews the clinical application of gene therapy for rare diseases such as blood diseases, neurodegenerative diseases and eye diseases. At present, gene therapy still has some technical problems and rare diseases are complicated and cannot be effectively treated. In the future, with further research and overcoming these problems, the application of gene therapy in rare diseases will make continuous breakthroughs and bring good news to mankind.

Keywords: gene therapy, rare diseases, gene editing, gene delivery.

1. Introduction

Rare diseases have a very low incidence and are mostly caused by congenital genetic defects. Most of the patients are children, and many of them are serious chronic diseases with a small number of patients. Different countries, organizations and regions have different definitions of rare diseases [1]. The European Union defines rare diseases as life-threatening or chronic and progressive diseases with a prevalence rate of less than 1/2000 and requiring special intervention [2]. In the *Rare Diseases Act 2022*, the United States defines rare diseases strictly according to their prevalence, that is, the number of patients in the United States is less than 200,000, and the prevalence rate is 1/1500 [2]. The World Health Organization defines rare diseases as having between 0.65% and 1% of the total population for a disease or lesion. However, according to "China Rare Disease Definition Study Report 2021", rare diseases are defined in China as diseases with an incidence less than 1/10000, prevalence less than 1/10000 and a number of patients less than 140,000 among newborn children [2].

There are a wide variety of rare diseases. Now there are about 7,000 rare diseases known. Although a single disease is very rare, the number of patients is about 300 million in the huge global population base, and there are about 20 million Chinese patients, with more than 200,000 new patients every year [3]. 80% of rare diseases are caused by congenital genetic defects and have genetic properties, and 80% of patients develop symptoms in childhood [3]. Families and society are looking forward to new treatment technologies, which is one of the greatest medical challenges of mankind.

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DOI: 10.54254/2753-8818/21/20230904

At present, the difficulty of diagnosis and treatment of rare diseases has attracted global attention, and there is a lack of effective treatment methods in the treatment of rare diseases in various countries. This paper reviews the clinical application of gene therapy for rare diseases such as blood diseases, neurodegenerative diseases and eye diseases. The emergence of gene therapy brings hope to patients with rare diseases, and mankind has made a further step in the biggest medical challenge. Gene therapy will promote the development of medical technology in the future, bringing hope to mankind.

2. Gene therapy strategy

Gene therapy is an emerging therapeutic method, which uses vectors to introduce genetic material into target cells to treat or prevent diseases by correcting or supplementing defective genes [3]. It has a good therapeutic prospect in the treatment of cancer and genetic diseases. In 1990, through therapeutic clinical trials in the United States, retrovirus vectors were used to introduce genes with correct coding into patients, restoring gene synthesis in patients, which initiated the first successful gene therapy [4]. Gene therapy for disease requires first identification of pathogenic genes, design of therapeutic genes through pathogenic genes, then specific genes into targeted cells, and finally evaluation of therapeutic efficiency. The selection of gene therapy strategies is crucial for the success of disease treatment. Different treatment strategies can be adopted according to different etiology and pathological changes [5]. To sum up, gene therapy can choose the treatment of diseases according to the changes in etiology and pathology through personalized strategies.

2.1. Gene delivery

Delivery of gene therapy requires vectors. DNA, RNA and protein are easily degraded or inactivated when encountering enzymes. Gene vectors protect DNA, RNA and protein and carry them to overcome multiple intracellular and external obstacles. Vector is particularly important in gene therapy. Vector of gene therapy includes viral vectors and non-viral vectors.

Retroviruses and adeno-associated viruses (AAV) are commonly used viral vectors in gene therapy [2]. Retroviruses belong to a class of RNA viruses, and lentiviruses are a special class of retroviruses that can carry large genes. Nowadays, they are often used as common vectors to deliver genes to hematopoietic stem cells [2]. AAV is a single stranded DNA parvovirus discovered in the 1860s [6]. The AAV vector used for in vivo gene therapy is modified from wild-type AAV. As a result of the natural evolution of the virus, it has high transfection efficiency, and the nucleic acid (DNA or RNA) of the virus itself is transferred to the host cell for replication [3].

With the development of materials and preparation techniques in recent years, non-viral vectors with low price, easy synthesis, easy purification, high transfection rate and low immunogenicity have become the best candidate for gene therapy. Non-viral vectors mainly include liposome vector, polymer vector, inorganic nanoparticle vector, and so on. These vectors can safely transport mRNA across the plasma membrane barrier to antigen-presenting cells [7].

2.2. Gene editing

Gene editing technology can accurately transform DNA sequences at the genome level. Through targeted double-strand breaks generated in DNA, cell DNA repair pathways can be activated to achieve the insertion, knockout and site-specific mutation of target genes, which is a precise therapy in a true sense [8]. Gene editing technology can deactivate or correct mutated genes in the genome, and edit defective genes into normal genes to achieve the purpose of treating diseases [2]. Traditional gene editing technology uses embryonic stem cells and homologous group technology to carry out site-specific modification of the genome [9].

Gene editing technology has a good application prospect in the treatment of genetic diseases and has become the hottest technology in the field of biology, and its application in the field of gene therapy has expanded from single-gene genetic diseases to the treatment of tumors, cardiovascular diseases, infectious diseases and other diseases [7]. However, there are drawbacks such as off-target effects [7].

DOI: 10.54254/2753-8818/21/20230904

2.3. Regulation of geneexpression or gene control

Gene therapy for some diseases requires not only the correct expression of genes, but also the realization of regulated expression of genes [9]. Commonly used small molecule drugs, including tetracycline regulation, sirolimus (rapamycin) regulation, etc., miRNA post-transcriptional regulation has also been applied to gene timing expression design.In recent years, more and more studies have been conducted on gene expression regulated by magnetic and photoregulation [9]. A safe and reliable regulated gene expression system is undoubtedly an important link in gene therapy technology, as well as an important embodiment of increasingly accurate gene therapy [9].

2.4. Gene replacement

In situ correction or replacement of mutant genes in the genome with normal genes is called gene replacement [9]. The main principle of this method is to use the site-specific recombination technology of genes to introduce a gene or expression element with normal function into the cells with abnormal function caused by gene mutation or other parts of the body with gene defects through the carrier, so as to supplement the missing function of the gene through the expression of complete proteins [9]. Theoretically, this method is the most ideal gene therapy strategy, but its disadvantage is that it is only applicable to the treatment of recessive pathogenic genes, such as a variety of hemophilia-related mutated genes, due to the influence of the recessive pathogenic genes [10-11]. For dominant pathogenic genes, functional supplement cannot cover up the influence of gene mutation [9].

2.5. The challenges of gene therapy

Although gene therapy has shown promise in clinical trials, it is also faced with multiple challenges. Compared with traditional drugs, gene therapy is an emerging treatment technology with rapid technological change and involves a variety of ethical and legal issues, especially safety risks [12]. Gene therapy drugs are mostly used to treat rare diseases, tumors and other diseases, and many patients are discouraged by their high price [7]. The production and purification methods of viral vectors are inefficient, and producing enough AAV vectors for clinical trials is a major challenge [3]. The current production and purification system has a series of problems such as difficult scaling up of production process, difficult purification, poor stability, etc. In addition to the complex preparation process of viral vectors, there are also problems such as packaging capacity limitation, insufficient gene expression conversion rate, poor immunogenicity, etc. [3].

Therefore, gene therapy will mainly solve these problems. For different diseases, gene therapy not only needs to be specific and efficient in targeting cells and tissues, but also needs to be able to accurately regulate [2]. Especially for complex diseases such as the expression of insulin genes in diabetes, it is necessary to include receptors that can sense blood sugar levels and determine how much insulin is produced based on blood sugar. And gene therapy needs to be accessible to patients, and the cost of preparation needs to be greatly reduced, from sky-high drugs to affordable drugs for most patients [7]. The development of gene therapy industry still needs to continue to improve the regulatory system, especially in terms of quality control, the establishment of mature third-party testing institutions should be accelerated, and the communication between pharmaceutical companies and drug regulatory agencies should be strengthened [7]. If these problems are solved through the joint efforts of scientific research organizations all over the world in the future, gene therapy, as a new treatment method, will take a historic step in the medical development of the world.

3. Clinical application of gene therapy for rare diseases

Gene therapy has made a lot of progress in the field of medical research, and its effectiveness and safety have been greatly improved. At present, it has achieved good results in the treatment of a variety of diseases. After the success of animal clinical trials, gene therapy began to enter human clinical trials [3]. With the development of diagnostic technology for the treatment of rare disease genes and the progress of gene editing technology, gene therapy has become one of the key research directions for the treatment

DOI: 10.54254/2753-8818/21/20230904

of rare diseases, which uses vectors to introduce genetic material into target cells for repair therapy and "one-time cure".

3.1. Blood diseases

Single-gene diseases affecting proteins and cells in the blood can be treated by gene backup or gene editing [3]. Hemophilia is a rare genetic disorder that causes blood clotting abnormalities. Bleeding from the joints or deep into the muscles can lead to continued dysfunction and even life-threatening bleeding. Therefore, hemophilia, as a single-gene disease, is very suitable to be cured by gene therapy [3]. Gene therapy provides a one-time treatment in which the missing functional gene is introduced so that the cell can synthesize clotting factors again. The functional gene can be introduced into the cell via a vector, usually a viral vector. The first clinical trial using AAV for hemophilia B by delivering clotting factor IX to muscle tissue showed a good safety profile, and the transgene persisted many years after administration [3].

3.2. Neurodegenerative diseases

Most monogenic diseases cause neurological symptoms, and many genetic mutations that cause neurological diseases have been identified [3]. Such as Alzheimer's disease, Parkinson's syndrome, amyotrophic lateral sclerosis, etc. Such neurodegenerative diseases are treated by inducing the formation and growth of neurites. AAV vector can transfer genes into neurons or other nerve cells through one-time administration and has long-term efficacy, providing great advantages for the treatment of central nervous system diseases with AAV [3].

So far, the safety and long-term effectiveness of AAV gene therapy has been confirmed in clinical studies on neurological diseases such as Parkinson's syndrome [13-14]. It shows that gene therapy has great potential in neurodegenerative diseases [3].

3.3. Eye diseases

Aav-mediated gene therapy has good applicability in eye diseases, because scientists have discovered genes responsible for a range of eye diseases. The good anatomy of the eye, with its limited and enclosed physical space, provides unique advantages for local delivery; The blood-eye barrier helps maintain the immune privilege of the eye and limits the immune response [15].

Gene therapy for eye diseases is also of great historical importance. In 2008, three independent groups demonstrated the safety and effectiveness of subretinal injection of AAV-RPE65 [16-17], gene therapy announced its return after more than a decade of silence [18]. Subsequently, the US Food and Drug Administration approved the first AAV gene therapy drug, Luxturna, for the treatment of Leber's congenital black eye syndrome [19].

At present, eye diseases treated with AAV that have entered phase I/II clinical trials include achromatosis (NCT02407678) and retinitis pigmentosa (NCT02556736). No serious complications related to AAV carriers have been found, and some patients have observed therapeutic effects. However, there are differences in visual effects after treatment, and the long-term efficacy is still uncertain. Some patients gradually decrease after reaching their peak treatment efficacy [20-22], possibly due to ongoing retinal degeneration or activation of innate immune responses [3].

3.4. Muscular disorders

Neuromuscular diseases are a group of genetic and acquired diseases that primarily affect one or more components of neuromuscular units, including motor neurons and skeletal muscles. Many neuromuscular diseases, such as Duchenne muscular dystrophy and spinal muscular atrophy, have clear genetic defects and single-gene disease properties, making gene therapy a promising treatment method [3]. AAV gene therapy has achieved remarkable efficacy in clinical trials of infantile spinal muscular atrophy [23]. Fifteen children who received a one-time intravenous infusion of AAV gene therapy had prolonged survival, improved motor function, and some could even walk [3].

DOI: 10.54254/2753-8818/21/20230904

Currently, clinical research is using different AAV serotypes and muscle-specific promoters to treat various neuromuscular diseases, such as Duchenne muscular dystrophy and X-linked myotube myopathy mentioned above [24]. Despite the promise of these gene therapy trials, challenges remain. Some patients develop an immune response to gene therapy, and systemic delivery of high doses of AAV can cause patients to experience transient and acute renal impairment with complement system activation.

3.5. Infectious diseases

Among infectious diseases, gene therapy mainly focuses on refractory diseases such as viral hepatitis, AIDS and SARS [12]. The introduction of protein genes that inhibit viral reproduction and promote viral death or nucleotide sequences that inhibit viral protein synthesis into target cells can reduce viral infection and proliferation in vivo to a certain extent [12]. For example, lentiviral vector-mediated CCR5 antibody expression can effectively inhibit HIV infection [25]. So gene vaccine has become a hot topic in the treatment of infectious diseases.

4. Current status of drug research and development for rare diseases

FDA, EMA, etc. have opened up special channels for rapid approval of orphan drugs, so that orphan drugs have the advantages of a high R&D success rate, short clinical trial cycle, low cost, fast approval, and high market price, which can effectively remedy the defect of fewer target patients; in addition, due to the lack of effective treatment drugs for most rare diseases so far [5]. Moreover, the urgent demand has led to a large development space and low market competition for orphan drugs, which has stimulated the enthusiasm of pharmaceutical companies [5].

5. Conclusion

Due to the complex types and many types of rare diseases [2], many rare diseases with serious diseases are still looking for more effective treatment methods. As an emerging treatment method, gene therapy is expected to solve the dilemma of human beings in the treatment of rare diseases. Gene therapy is still in its early stage and many problems need to be solved, but it can establish personalized strategies. One-time treatment of diseases has a profound impact on the development of rare disease treatment, so there is a lot of research space for gene therapy of rare diseases in the future. Gene therapy is a medical technology with great potential and challenges, which can offer hope and solutions for many diseases that cannot be treated by other methods. Early detection and diagnosis of rare diseases can be realized through genetic diagnosis, and more and more patients with rare diseases are expected to be cured by combining gene editing with personalized medicine. The extensive application of big data and artificial intelligence in medical treatment will also greatly improve the quality of life of patients with rare diseases [5].

With the improvement of the safety and effectiveness of viral vectors, gene therapy has made breakthroughs in many genetic diseases [2], bringing light to patients with rare diseases. The application of gene editing technology makes the original disease-causing genes too large to exceed the packaging limits of viral vectors, so that the gene delivery method cannot be used to make breakthroughs, and the gene therapy research on brain and other technically difficult tissues has also made breakthroughs [2]. However, in order to apply gene therapy to the clinical treatment of more widespread but inherited rare diseases, many problems still need to be solved, such as immune rejection, gRNA recognition range, insertion mutagenesis, genotoxicity caused by off-target effect, ethical issues and high but expensive treatment costs [2]. With the continuous development of future research and continuous solution to problems, it is expected that the application of gene therapy in rare diseases will make continuous breakthroughs in the near future, bringing good news to mankind.

DOI: 10.54254/2753-8818/21/20230904

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Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230905

A review of the recent research progress on risk factors of lung cancer

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Abstract. As a malignant tumor, Lung cancer has the highest morbidity and mortality rate worldwide. Various risk factors can cause lung cancer, of which the etiological structure is complicated and the specific mechanism has not been clarified yet. In this paper, the author reviews the recent research progress on risk factors of lung cancer, and conclusions can be drawn that smoking is currently the main risk factor causing lung cancer. The risk of developing lung cancer for smokers is 22 times higher than that of non-smokers. Another risk factor is known as indoor air pollution such as formaldehyde released by furniture and household air pollution (HAP). The majority of recent investigations confirm that indoor air pollution is connected to the development of lung cancer. In addition, previous research has also demonstrated a connection between lung cancer and a number of respiratory illnesses, especially chronic obstructive pulmonary disease (COPD). Moreover, a family history of tumors can also be a risk factor, for both men and women are at an increased risk of developing lung cancer if there is a history of the disease in close relatives. What is more, recent research also proves that the intake of fruits and vegetables can lower the incidence of lung cancer.

Keywords: lung cancer, risk factors, etiology, research progress.

1. Introduction

Based on epidemiological research, the interaction of genetics and environments is still the major risk factor that leads to the occurrence and development of lung cancer. At present, in addition to smoking-associated factors, it is difficult to explain how the incidence of lung cancer is related to genetics, food, chronic lung disorders, environmental and occupational exposure, and poor habits [1]. There were 18.1 million new cases of cancer worldwide in 2018, according to GLOBOCAN 2018, a report from the International Agency for Research on Cancer, a division of the World Health Organization (WHO). Based on the data collected on 36 types of cancer in 185 nations, 9.6 million people annually die from cancer, and Asian people represent over half of all cancer cases. Besides, Lung cancer has the highest incidence and mortality rates among mixed-gender populations (11.6% of all cases and 18.4% of all cancer-related deaths, respectively) [2]. In 2014, there were 626,000 lung cancer fatalities and 781,000 new cases, according to the National Tumor Registration Center. Lung cancer continued to be a leading cause of death for both sexes between 2000 and 2014, ranking first among malignant tumors during that time [3]. The total incidence of lung cancer also exhibited an upward trend during that time period.

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DOI: 10.54254/2753-8818/21/20230905

This paper discusses recent findings on a few risk factors for lung cancer, including smoking, indoor air pollution, a history of lung chronic disease, a family history of the disease, diet, and nutrition, so as to offer a theoretical foundation for early detection, diagnosis, and treatment of lung cancer.

2. Smoking

According to the research conducted both domestically and abroad, smoking is currently the main reason for the development of lung cancer. According to a 2017 estimate from an American study, 19.3% of American adults (about 47.4 million) used tobacco. 86.7% (about 41.1 million) of them used combustible tobacco products and 19.0% (about 9 million) of them used two or more types [4]. WHO noted in the 2019 World No Tobacco Day promotion that 1.2 million people die annually from smoking-related lung cancer; compared to non-smokers, smokers are at a 22 times greater risk of developing lung cancer [5].

Based on the identification and quantitative analysis of the risk factor data of lung cancer in the elderly population in the U.S. Behavioral Risk Factor surveillance system, Chen et al. [6] showed in their study that the main risk factor for lung cancer in men aged 65 and over is the frequency of smoking. Significant risk factors for lung cancer in women aged 65 and over include the length of smoking cessation and whether they have at least 100 cigarettes smoked. It has been determined that smoking-related variables are the primary causes of lung cancer in older people. Environmental tobacco contamination poses a serious danger of lung cancer. Lung cancer risk was 30 percent higher for nonsmokers exposed to second-hand smoke at home or work [4].

Environmental tobacco smoke (ETS) is linked to dose exposure in women with lung cancer, according to domestic studies in Shenyang [7] and Tangshan [8] in China. This association has been comprehensively reviewed. The primary risk factor for lung cancer in women is high-dose ETS exposure, particularly over a 20-year period.

Another major research focus on the effects of environmental tobacco pollution is the link between exposure to third-hand smoke (tobacco residue) and lung cancer. According to a study by Moon et al. [9], third-hand smoke is easily inhaled by people who frequently use public transportation, saunas, bars, and Internet cafes. It is also easily inhaled by people who have recently gone through a divorce or a loss, who have low levels of education, or who have smokers in their homes.

Currently, only in vitro and animal studies have shown the impact of third-party smoking on the body; further research is needed to determine whether it can induce lung cancer [10].

3. Indoor air pollution

Household air pollution (HAP), formaldehyde released by furniture, and nitrogen oxides created by gas installations are all examples of indoor air pollution. The majority of recent investigations confirm that HAP is connected to the incidence of lung cancer. HAP is mostly caused by the use of biomass (including wood, charcoal, straw, and animal waste), coal, and other solid fuels for heating or cooking in small spaces in China, particularly in the northern regions.

The lung cancer mortality rate in Xuanwei City, Yunnan Province is among the highest in China. The substantial pollution brought by burning bituminous coal in a nearby indoor fireplace without a chimney has been demonstrated in prior research to be the main reason for the high mortality rate of lung cancer in the Xuanwei area. Liu et al. [11] discovered that although the incidence of lung cancer in Xuanwei is still high and may be related to tobacco exposure, bituminous coal use, and occupational exposure of the Xuanwei population, the current distribution of bituminous coal use is no longer consistent with the distribution of high, middle, and low incidence areas of lung cancer.

According to a 2020 study by Liu et al. [12], tobacco exposure leads to a more significant risk of developing lung cancer compared to indoor air pollution since no coal was used more than 30 years ago. They discovered that indoor air pollution had a strong lagged effect on lung cancer risk in Xuanwei more than 30 years ago. According to studies, smoking or exposure to second-hand smoke can lessen the impact of indoor air pollution on lung cancer by up to 18 to 30%.

DOI: 10.54254/2753-8818/21/20230905

Cooking oil fumes can also cause lung cancer in women, regardless of the impact of smoking, according to Jia et al. [13], who investigate the association between cooking and lung cancer in adults. Additionally, improper ventilation when cooking may raise the risk of lung cancer.

4. Pulmonary history

Previous research has demonstrated a connection between lung cancer growth and a number of respiratory illnesses. COPD has been long considered one of the major risk factors for lung cancer.

In their investigation, Yang et al. [14] identified the shared etiological spectrum of COPD and lung cancer and hypothesized that COPD acts as a catalyst for the growth of lung cancer. According to studies, when smoking (or high cumulative smoking) and burning biomass (coal, wood, etc.), people with pre-existing COPD have a substantially greater risk of having lung cancer than those without COPD. Additionally, smoking, passive smoking, burning biomass, and the incidence of lung cancer are all mediated by COPD.

However, the study of Sandelin et al. [15] came to a different conclusion. Unlike some previous studies that suggested both COPD and asthma as independent risk factors for lung cancer, this large cohort study based on 19894 COPD patients concluded that the hazard ratio for patients with COPD complicated with asthma to have lung cancer was lower than that for patients with COPD alone.

5. A family history of tumors

Numerous earlier investigations on lung cancer have found that in non-smoking lung cancer patients, genetic sensitivity is connected to the phenomena of cancer family clustering. According to Lin et al. [16], people with a history of lung cancer or any other type of cancer are at a higher risk of having the disease, particularly if their mother had the disease in the past.

According to Yoshida et al. [17], both men and women are at an increasing risk of developing lung cancer if there is a disease history in close relatives. An elevated risk of lung adenocarcinoma in women was found to be significantly correlated with a parent's history of the disease; however, this correlation was only found in former smokers. For men with a family history of the disease, the risk of developing small-cell lung cancer and lung adenocarcinoma was considerably higher.

6. Nutrition and diet

Dietary pattern analysis is a new method for examining how diet affects cancer risk. It does not focus on specific nutrients or foods, but rather on the overall impact of a mixed diet.

According to Wang et al. [18], the intake of fruits and vegetables can lower the rate of developing lung cancer, and this link was more pronounced in women. According to a controlled investigation by Deneo-Pellegrini et al. [19] on 300 patients with lung squamous cell carcinoma and 600 patients in the control group, increased meat consumption dramatically raised the possibility of having lung cancer.

In a study by Mahabir et al. [20], 482,875 cancer-free participants participated in a 7-year questionnaire follow-up survey, during which time 7052 of them received a lung cancer diagnosis. The total intake of copper was protective for smokers and adenocarcinoma patients; the total intake of magnesium increased the rate of lung cancer in men and current smokers; the total intake of iron was inversely related to the incidence of lung cancer in women. The researchers found no evidence that the total intake of calcium, magnesium, iron, copper, selenium, or zinc (dietary plus supplements) was associated with the rate of lung cancer among all participants. In terms of dietary minerals, higher calcium intake was linked to a decreased risk of lung cancer in women, however, minerals from dietary supplements were not.

Wakai et al. [21] conducted an analysis using data from four cohort studies with more than 200,000 participants and more than 1700 lung cancer patients. The result findings revealed that the intake of fruits and vegetables can lower the mortality rate for lung cancer patients, particularly when they consume moderate amounts of fruits.

DOI: 10.54254/2753-8818/21/20230905

7. Conclusion

One of the major malignancies that pose the greatest threat to human life and health worldwide has always been lung cancer. The timely updating and investigation of lung cancer risk factors are crucial for the prevention and control of the occurrence and progression of lung cancer in light of the growth and development of society. Lung cancer is clearly influenced by smoking, second-hand smoke, and environmental tobacco exposure. There is a correlation between the occurrence of lung cancer and a history of lung-related disorders, indoor air pollution, family history of cancer, nutrition, and diet, but more research is still needed to determine the exact etiology. Making dietary adjustments is essential to lowering the risk of lung cancer, in addition to quitting smoking, lowering exposure to indoor pollutants, and raising health awareness among those with a family history of malignancies and lung cancer. Future multi-center, large-sample research is required to precisely determine the effect of diet and dietary practices on the risk of lung cancer and to motivate health professionals to counsel the populace on healthy diets. Lung cancer occurs as a result of a variety of elements acting together, in addition to those mentioned above, and its etiology includes a broad spectrum of causes. More in-depth exploration still needs to be done by being combined with a variety of disciplines, so as to provide scientific guidance for the prevention of lung cancer.

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