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Preface

The 4th International Conference on Biological Engineering and Medical Science (ICBioMed 2024) is an annual conference focusing on research areas including biological engineering, biological sciences, biomedical engineering and bioinformatics, basic science of medicine and 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, biological sciences, biomedical engineering and bioinformatics, basic science of medicine and clinical and public health.

This volume contains the papers of the 4th International Conference on Biological Engineering and Medical Science (ICBioMed 2024). Each of these papers has gained a comprehensive review by the editorial team and professional reviewers. Each paper has been examined and evaluated for its theme, structure, method, content, language, and format.

Cooperating with prestigious universities, ICBioMed 2024 organized three workshops in Sydney, Auckland and Lahore. Dr. Byeongsang Oh chaired the workshop “The Role of Gut Microbiome in Cancer”, which was held at University of Sydney. Dr. Alan Wang chaired the workshop “Workshop on Intelligent Medical Data Analysis for Precision Medicine”, which was held at University of Auckland. Dr. Ghulam Yaseen chaired the workshop “Computational Proteomics in Drug Discovery and Development from Medicinal Plants”, which was held at University of Education.

Besides these workshops, ICBioMed 2024 also held an online session. Eminent professors from top universities worldwide were invited to deliver keynote speeches in this online session, such as Dr. Alan Wang from University of Auckland and Dr. Byeongsang Oh from University of Sydney. They have given keynote speeches on related topics of biological engineering, biological sciences, biomedical engineering and bioinformatics, basic science of medicine and 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 2024, editors and reviewers who have guaranteed the quality of papers with their expertise, and the committee members who have devoted themselves to the success of ICBioMed 2024.

Dr. Byeongsang Oh

Dr. Alan Wang

General Chairs of Conference Committee

Workshops

Workshop – Sydney: The Role of Gut Microbiome in Cancer

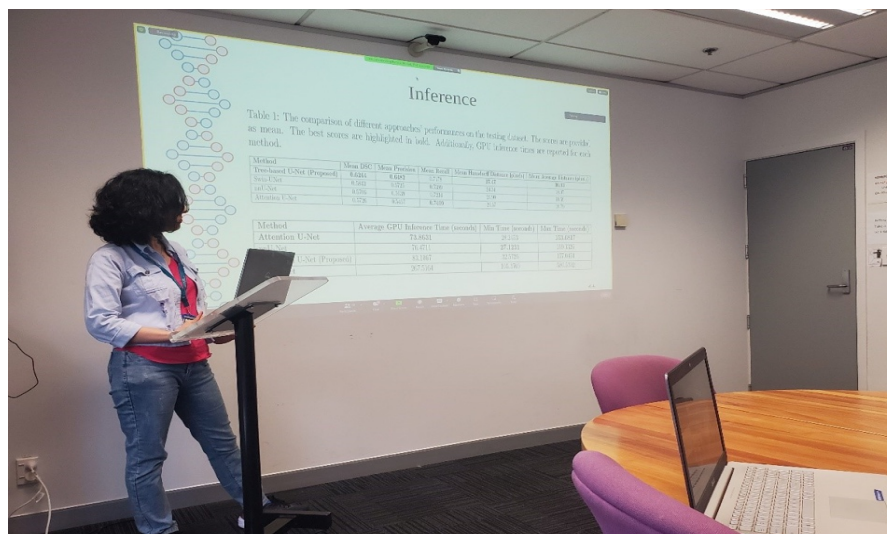


October 25th, 2024 (UTC +11)

Royal North Shore Hospital, University of Sydney

Workshop Chair: Dr. Byeongsang Oh, Associate Professor in University of Sydney

Workshop – Auckland: Workshop on Intelligent Medical Data Analysis for Precision Medicine



September 13th, 2024 (UTC +13)

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

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

Workshop – Lahore: Computational Proteomics in Drug Discovery and Development from Medicinal Plants



November 7th, 2024 (UTC +5)

Department of Botany, Division of Science and Technology, University of Education

Workshop Chair: Dr. Ghulam Yaseen, Assistant Professor in University of Education

The 4th International Conference on Biological Engineering and Medical Science

ICBioMed 2024

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Association between transferrin receptor and urine cobalt: NHANES 2015-2020

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Abstract. The transferrin receptor protein 1 (TfR1) is a critical cellular membrane protein. It orchestrates the internal transport and metabolism of iron, a vital process for intracellular functions. Additionally, TfR1 has the capacity to bind with metals beyond iron, including cobalt and manganese; however, the specific mechanisms of this binding and subsequent interactions with other proteins remain elusive. This study delves into the potential correlation between TfR1 levels in humans and the presence of cobalt in urine. Drawing on data from 3,424 participants across three survey cycles spanning two years each, this paper meticulously contrasts the clinical features of individuals across diverse TfR1 and urinary cobalt levels. Through the lens of both univariate and multivariate linear regression analyses, the research uncovers an inverse connection between TfR1 concentrations and urinary cobalt, post exclusion of participants with incomplete biochemical profiles. This inverse relationship could shed light on the complex interplay between TfR1 and metal ions within the body, providing a deeper understanding that may inform future studies and potential clinical applications. In the multivariate linear regression analysis, it is found that with the increasing of TFR1 quartiles, the proportion of obese participants ($P < 0.01$), high level blood manganese, high cobalt level in urine and blood ($P < 0.01$) increased gradually. This association remained significant in sensitivity analysis, while in the stratified analysis, above association was not significant in men with samples aged 65 and over. Transferrin receptor protein levels might be positively associated with urinary cobalt in the general U.S. crowd.

Keywords: Transferrin receptor protein (TfR1), Cobalt, The National Health and Nutrition Examination Survey (NHANES).

1. Introduction

In people's blood, most of the iron is carried by a protein called transferrin. This protein is especially important for the development of red blood cells in the bone marrow, as these cells heavily rely on iron that is attached to transferrin. They grab onto this iron using a special structure on their surface known as transferrin receptor 1 (TfR1), which red blood cells have in abundance [1]. The transferrin, with its iron cargo, binds to TfR1. TfR1 is quite the specialist; it is a protein built for the job, being a 97-kDa homodimer (a structure made of two identical subunits) that is particularly good at holding onto transferrin carrying two iron atoms [2]. After the iron is released inside the cell, it is transformed into a form that the cell can use, but TfR1 is not done yet, though; it is recycled back to the cell surface to fetch more iron [3]. An interesting point to note is the different forms iron takes inside versus outside of cells.

Inside cells, iron is usually in the Fe^{2+} form, while outside, it is in the Fe^{3+} form [4]. This difference is likely to play a role in keeping the cell's internal environment stable and suitable for its many biochemical processes, reflecting the cell's ability to maintain a more reduced, or electron-rich, environment compared to the outside of the cell [5].

Cobalt, although only needed in tiny amounts, plays a crucial role in the human body's health and functionality [6]. One of its key jobs is to help stabilize proteins called hypoxia-inducible transcription factors [7]. When these factors are stable, people's bodies can produce more erythropoietin, a hormone that signals the production of red blood cells [8]. This process is essential for maintaining healthy oxygen levels in people's tissues and organs, showcasing cobalt's vital part in keeping people healthy and well-oxygenated.

For a metal to follow the iron acquisition pathway, many different conditions are required [9]. Among these metals, cobalt can be translocated by transferrin receptors, competing with iron [10]. The study compares the clinical attributes of subjects across varying groups categorized by transferrin receptor protein levels and urinary cobalt concentrations. Both univariate and multivariate linear regression analyses were utilized to assess the relationship between transferrin receptor protein levels and urinary cobalt concentrations. The specificity of the correlation between cobalt and transferrin receptor content is confirmed by analyzing the correlation between cobalt and manganese content in urine, blood, and transferrin receptor content.

This paper aims that the findings could enable the use of urinary cobalt levels as a diagnostic tool for anemia, neurodegenerative conditions, and other ailments associated with human transferrin receptor levels. The project anticipates that this data may offer valuable insights into the therapeutic strategies of these disorders.

2. Methods

2.1. Data source and study population

The Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) is a pivotal biennial assessment of America's health and nutrition trends. This systematic survey encompasses comprehensive interviews at participants' homes, followed by thorough health evaluations in mobile examination units. These units are fitted with the requisite medical apparatus to facilitate physical and laboratory examinations by proficient medical staff. Rigorous protocols for participant consent and data anonymity are strictly adhered to in accordance with ethical standards set by the National Center for Health Statistics.

For the current study, the dataset was curated from three NHANES cycles spanning 2015–2016, 2016–2017, and up to March 2020. The cohort was carefully filtered to omit any individuals with incomplete blood and urine analyses or unavailable TFR1 data, culminating in a robust sample of 3,424 subjects for the analysis (see Figure 1).

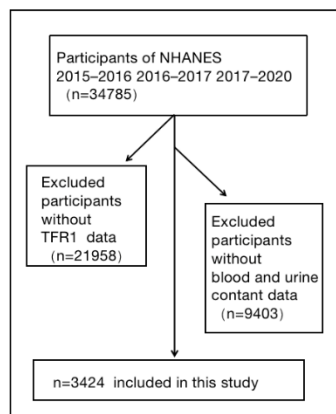


Figure 1. Flow chart of the screening process for the selection of eligible participants.

2.2. Variables

The method principle for measurement of soluble transferrin receptor (sTfR) is a particle-enhanced immunoturbidimetric assay that uses Roche kits on the Cobas® c501 clinical analyzer. The lower limit of detection for the transferrin receptor is 0.500 mg/L. This method directly measures the cobalt level in urine specimens using mass spectrometry after a simple dilution sample preparation step. The lower limit of detection for urinary cobalt is 0.023 (ug/L). Analytes with analytic results below the lower limit of detection are divided by the square root of 2.

2.3. Covariates

Potential confounding factors in this study include age and BMI (normal: below 25 kg/m²; overweight: between 25 and 30 kg/m²; obesity: ≥ 30 kg/m²). The invasion of the hepatitis C virus into the human body is assisted by the transferrin receptor, taking as a covariate whether or not the subject has ever had hepatitis C. The transferrin receptor is closely related to blood erythrocytes, so this project took the patient's erythrocyte count and hemoglobin content. Previous studies have demonstrated that manganese is transported by transferrin receptors, so the project selected both urine and blood manganese levels as covariates with blood cobalt levels.

2.4. Statistical analysis

All statistical analyses are calculated using R (version 4.2.2). The normality of continuous variables is tested with the Kolmogorov-Smirnov normality test. Normally distributed variables are described as the mean $\pm$ standard deviation, and nonnormally distributed continuous variables are described as the median (interquartile range). The median values among different transferrin receptor and urinary cobalt groups were compared with the Mann-Whitney U test and Kruskal-Wallis test. The chi-square test was adopted to compare the percentages of categorical variables among different transferrin receptor and urinary cobalt groups. The Bonferroni test is used for the intergroup comparison. This project evaluates the correlation between each independent variable and urinary cobalt by using univariate and multivariate linear regression models. Because the distribution of the transferrin receptor is skewed right, it is log-transformed in the regression analysis. With the transferrin receptor as the dependent variable, regression coefficients, standard errors, and P values are determined.

In addition, stratified analyses by age (group 1: $18 \leq \text{age} < 45$, group 2: $45 \leq \text{age} < 65$, group 3: $\text{age} \geq 65$), BMI (normal, overweight, and obesity), history of Hepatitis C are performed to examine the association between urinary cobalt and log-transformed transferrin receptor. Urinary cobalt distribution is categorized into normal and excessive groups based on 0.1 (1ug/l). This project analyzes the multicollinearity of the multivariate linear regression. A two-sided $P < 0.05$ is considered statistically significant.

3. Results

A total of 3424 participants are included in this study. The mean age is 38.57, the mean transferrin receptor level is 42.18 nmol/L, and the mean blood cobalt level is 0.68 ug/L. As seen in Table 1, transferrin receptor levels are categorized based on transferrin receptor quartiles: Q1 ($\text{TFR1} \leq 29.9$ nmol/L), Q2 ($29.9 < \text{TFR1} \leq 36.3$ nmol/L), Q3 ($36.3 < \text{TFR1} \leq 45.0$ nmol/L), and Q4 ($\text{TFR1} > 45.0$ nmol/L). A Bonferroni test is adopted for intergroup comparison, and this project finds that the proportion of obese participants ($P < 0.01$), the level of blood manganese, and the cobalt level in urine and blood ($P < 0.01$) increase gradually with the increasing of TFR1 quartiles. It is interesting that the levels of cobalt and manganese in urine and blood are different in relation to the transferrin receptor composition, even though both of them have been shown to be transported by the transferrin receptor.

Table 1. Clinical characteristics of the study population disaggregated by quartiles of the transferrin receptor level.

	Transferrin Receptor Level (by quartile)				p	test
	Q1	Q2	Q3	Q4		
n	866	857	852	849		
age (median [IQR])	37.00 [25.00, 51.00]	35.00 [20.00, 53.00]	37.00 [21.00, 55.25]	36.00 [19.00, 48.00]	0.028	nonnorm
BMI (mean (SD))	3.00 (0.00)	3.05 (0.23)	3.08 (0.79)	3.06 (0.24)	0.909	
URDUPBLC (median [IQR])	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.423	nonnorm
blood manganese (median [IQR])	167.09 [137.24, 204.68]	174.19 [140.88, 216.42]	180.75 [146.16, 226.80]	210.96 [168.00, 275.21]	<0.001	nonnorm
urine volume (median [IQR])	79.00 [44.00, 133.75]	84.00 [47.00, 148.00]	75.00 [43.00, 135.00]	81.00 [47.00, 139.00]	0.013	nonnorm
Transferrin receptor content (mean (SD))	2.19 (0.25)	2.81 (0.16)	3.40 (0.21)	5.94 (3.61)	<0.001	
Manganese in urine (mean (SD))	0.18 (0.60)	0.15 (0.21)	0.17 (0.28)	0.19 (0.60)	0.324	
Lead in urine (mean (SD))	0.43 (0.62)	0.41 (1.01)	0.40 (0.50)	0.40 (0.43)	0.715	
Cobalt in urine (mean (SD))	0.49 (0.63)	0.60 (1.34)	0.61 (0.69)	1.02 (1.50)	<0.001	
Red blood cell content (median [IQR])	4.58 [4.30, 4.84]	4.66 [4.38, 4.96]	4.66 [4.39, 4.98]	4.70 [4.36, 4.96]	<0.001	nonnorm
hemoglobin content (median [IQR])	13.80 [13.10, 14.70]	13.70 [13.10, 14.80]	13.60 [12.80, 14.50]	12.80 [11.50, 13.70]	<0.001	nonnorm
BMI_category (%)					<0.001	
<25	358 (41.3)	328 (38.3)	275 (32.4)	252 (29.7)		
25-30	243 (28.1)	243 (28.4)	232 (27.3)	211 (24.9)		
>=30	265 (30.6)	286 (33.4)	343 (40.4)	386 (45.5)		
HCV history = Yes (%)	8 (0.9)	11 (1.3)	3 (0.4)	10 (1.2)	0.188	
UA_quantile (%)					<0.001	
Q1	866 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Q2	0 (0.0)	857 (100.0)	0 (0.0)	0 (0.0)		
Q3	0 (0.0)	0 (0.0)	852 (100.0)	0 (0.0)		
Q4	0 (0.0)	0 (0.0)	0 (0.0)	849 (100.0)		
age_cat (%)					0.013	
<45	564 (65.1)	541 (63.1)	532 (62.4)	579 (68.2)		
45-65	213 (24.6)	206 (24.0)	190 (22.3)	175 (20.6)		
>=65	89 (10.3)	110 (12.8)	130 (15.3)	95 (11.2)		

The clinical characteristics of participants with different urinary cobalt levels are shown in Table 2. It is found that there is a strong linear relationship between the transferrin receptor and urinary cobalt ($P<0.001$), and a higher level of urinary cobalt is associated with a higher hemoglobin and red cell level in the blood ($P<0.01$), which is closely associated with the presence of transferrin receptors. Besides,

there is a strong correlation between urine and blood cobalt levels ($P<0.001$). Samples with low blood cobalt levels are older (38 versus 29 years old) ($P<0.01$), and there is no strong linear relationship between obesity and urinary cobalt levels.

Table 2. The clinical characteristics of participants with different urinary cobalt levels.

	Cobalt Levels (high/normal)		p	test
	high	normal		
n	589	2835		
age (median [IQR])	29.00 [18.00, 43.00]	38.00 [22.50, 55.00]	<0.001	nonnorm
BMI (mean (SD))	3.08 (0.28)	3.04 (0.35)	0.734	
BMXHT (median [IQR])	162.80 [157.50, 168.50]	163.40 [157.55, 170.20]	0.048	nonnorm
Manganese in blood (median [IQR])	216.97 [169.46, 279.22]	175.83 [141.43, 219.88]	<0.001	nonnorm
cobalt in blood (median [IQR])	6.62 [4.24, 9.41]	2.55 [2.21, 3.05]	<0.001	nonnorm
urine volume (median [IQR])	73.00 [45.00, 116.00]	82.00 [45.00, 142.00]	0.001	nonnorm
Transferrin receptor content(mean (SD))	5.00 (4.08)	3.28 (1.55)	<0.001	
Manganese in urine (mean (SD))	0.21 (0.30)	0.16 (0.49)	0.023	
URXUPB (mean (SD))	0.66 (1.29)	0.36 (0.44)	<0.001	
cobalt in urine(mean (SD))	1.95 (2.27)	0.41 (0.24)	<0.001	

Then this project makes more exploration of the differences between manganese and cobalt. Clinical characteristics of the study population in different urinary manganese level groups (0.1ug/l) and blood manganese level groups (200nmol/L) are shown in Table 3. Interestingly, the project finds that there is no significant correlation between the urinary manganese level and the red blood cell and hemoglobin level ($P<0.01$), and there is a positive correlation between blood manganese levels and hemoglobin levels ($P<0.01$). However, there is a linear relationship between manganese levels in urine and blood.

Table 3. Clinical characteristics of the study population in different urinary manganese level groups (0.1ug/l) and blood manganese level groups (200nmol/L).

	Urinary Manganese Level Groups (0.1ug/l)		p	test
	high	normal		
n	1176	2248		
age(median [IQR])	35[19.00,52.00]	37[22.75,52.00]	0.057	nonnorm
Transferrin receptor content(mean (SD))	43.64(28.83)	41.42(26.13)	0.012	
Manganese in blood (median [IQR])	186.02[148.53,238.72]	179.65[144.66,227.53]	0.023	nonnorm
cobalt in urine(mean (SD))	0.93(1.25)	0.55(1.03)	P<0.001	
hemoglobin content(median [IQR])	13.50[12.80,14.50]	13.50[12.70,14.50]	0.51	nonnorm
Red blood cell content(median [IQR])	4.66[4.38,4.94]	4.62[4.34,4.94]	0.115	nornorm
blood manganese level groups (200nmol/L)				
	high	normal	p	test
n	1176	2248		
age(median [IQR])	34.00[20.00,46.00]	37.50[22.00,58.00]	P<0.001	nonnorm
Transferrin receptor content(mean (SD))	50.77(38.63)	36.84(13.75)	P<0.001	

Table 3. (continued)

cobalt in urine(mean (SD))	0.83(1.21)	0.59(1.06)	P<0.001	
hemoglobin content(median [IQR])	13.30[12.40,14.30]	13.70[12.90,14.60]	P<0.001	nonnorm
Red blood cell content(median [IQR])	4.65[4.38,4.92]	4.63[4.34,4.95]	0.411	nornorm

Back to the linear relationship between transferrin receptors and urinary cobalt levels, this project predicts the log-transformed transferrin receptor level through univariable and multivariate linear regression analysis (see Table 4). Both in univariable and multivariate linear regression, it is found that the increasing age ($P<0.01$) is associated with the declining transferrin receptor level while the manganese level in blood ($P<0.01$), the cobalt level in urine and blood ($P<0.01$), and the red blood cell and hemoglobin level ($P<0.01$) are associated with increasing transferrin receptor levels.

Table 4. Univariable and multivariate linear regression analysis predicting log-transformed transferrin receptor level.

Variable	Univariable Linear Regression Analysis				Multivariable Linear Regression Analysis		
	R2	B	SE	P value		B	SE value
Manganese level in urine	-0.0003	-0.0036	0.0145	0.802		-0.0207	0.0107 0.0535
lead level in urine	-0.0001	-0.0071	0.0098	0.475		0.0063	0.0074 0.3966
Arsenic level in urine	0.0001	0.0001	0.0001	0.286		0.0002	0.0001 0.0049
Cobalt level in urine	0.0419	0.0712	0.0058	<0.001		0.0315	0.0045 <0.001
hemoglobin level	0.0024	0.044	0.0145	0.0024	Adj.R2 0.4492	-0.1746	0.0045 <0.001
Red blood cell count	0.0441	0.044	0.01447	0.0023		0.3807	0.0139 <0.001
Urine volume	0.0003	0.0001	0.0001	0.15		0.0001	0.0001 0.0779
Manganese level in blood	0.1744	0.0021	0.0001	<0.001		0.0011	0.0001 <0.001
HCV	-0.0003	-0.0107	0.0694	0.878		0.0214	0.0514 0.6771
BMXBMI	0.0124	0.0057	0.0008	<0.001		0.0019	0.0007 0.0054
RIDAGEYR	0.0015	-0.0008	0.0003	0.0128		0.0018	0.0003 <0.001

Revisiting the analysis on the correlation between transferrin receptors and urinary cobalt concentrations, the study applies both univariate and multivariate linear regression models to forecast the levels of the log-transformed transferrin receptor. As shown in Table 5, the findings consistently indicate that an increase in age is linked to a decrease in transferrin receptor levels ($P<0.01$), while higher concentrations of manganese in the blood ($P<0.01$), as well as elevated cobalt levels in both urine and blood ($P<0.01$), along with increased red blood cell and hemoglobin levels ($P<0.01$), are associated with an upsurge in transferrin receptor levels.

In detailed subgroup analyses tailored for multivariate linear regression, specifically predicting the log-transformed levels of transferrin receptor, the study observes that the connection between transferrin receptor levels and cobalt concentrations remains statistically significant across different BMI categories and histories of Hepatitis C Virus (HCV) infection. However, this association does not hold statistical significance in the subgroup of participants aged 65 and above, indicating potential age-related disparities in the biochemical interactions between transferrin receptors and cobalt levels.

Table 5. Stratified analyses by selected characteristic, for multivariate linear regression analysis predicting the log-transformed transferrin receptor level.

Variable	Multivariable Linear Regression Analysis		
	B(Cobalt level)	SE	p value
Age (years)			
18	0.2356	0.0115	<0.001
45	0.0435	0.011	0.0001
Age>65	0.0012	0.0067	0.8564
BMI(kg/m2)			
BMI<25	0.2189	0.0169	<0.001
25	0.0354	0.0074	0.0001
BMI>30	0.0879	0.0106	<0.001
Suffered HCV			
Yes	0.0338	0.0138	0.0213
No	0.0782	0.0063	<0.001

4. Conclusion

This investigation synthesizes the findings from three biennial survey cycles spanning from 2015 to 2020, involving 3,424 participants, to explore the dynamics of transferrin receptor (TfR) levels in relation to urinary cobalt concentration. Utilizing both univariate and multivariate linear regression analyses, the study identifies a positive correlation between TfR levels and urinary cobalt. However, when dissecting the data further, this association does not hold for participants aged 65 and above. Additionally, the research underscores a strong linkage between urinary cobalt levels and hemoglobin and red blood cell counts, indicators that are closely tied to TfR presence. In contrast, while no significant relationship is found between urinary manganese levels and hemoglobin or red blood cell counts, an affirmative correlation emerges between blood manganese levels and hemoglobin, offering insights into the nuanced interplay of metal ions and biomarkers within the human body. Transferrin receptors have received much attention in popular fields such as Ferroptosis and iron metabolism. However, the relationship between the processes of iron metabolism and the total body substance metabolism is still unclear. Through the project, this correlation relationship can be indicative of human transferrin receptor levels in terms of urine biopsy data. It also indicates directions for the relationship between iron metabolism and human substance metabolism: the bioactive role of metallic elements.

The work also raises the question of why cobalt levels in urine correlate with transferrin receptor levels, while manganese, for example, does not. This may possibly related to the competition between cobalt and iron, resulting in spurious increasing transferrin receptor levels. Metal elements interact with the transferrin receptor through different mechanisms.

However, based on a cross-sectional design, this study can only explore the association between urine cobalt levels and transferrin receptor levels, and it cannot verify causality. The association the project investigated in this study is biologically plausible, and further large-scale prospective studies are required to confirm the causal relationship. There are some questions to be solved in the future, for instance, why there is a strong correlation between urinary cobalt levels and transferrin receptor levels. Further exploration of the causes of the differences in different metallic elements and mechanism studies are needed.

In sum, this research underscores a positive link between transferrin receptor levels and urinary cobalt concentrations within the U.S. populace. This finding holds promise for leveraging urinary cobalt as a biomarker for diagnosing conditions such as anemia, neurodegenerative diseases, or other disorders associated with transferrin receptor fluctuations. Ultimately, it is hoped that these insights can inform and enhance therapeutic strategies for these ailments.

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Ketogenic diet and its treatment for brain and other nervous system cancers

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Abstract. A growing body of empirical evidence indicates the paramount importance of nutrition in cancer treatment, especially glioma. Among the various strategies proposed to enhance traditional anticancer therapy, ketogenic diets (KD) have emerged as a noteworthy approach. In this review, seven papers highly relevant to the effects of ketogenic diet on glioma are selected and the factors involved are summarized. A Google Scholar search with the subject terms “ketogenic diet” and “brain cancer” or “ketone bodies” and “glioma” results in the selection of seven papers highly relevant to the topic. Animals injected with tumor cells or patients diagnosed with glioma, which had received a ketogenic diet for at least ten days, are included in the analysis. The metabolism-related measurements are also taken. The seven papers show that the ketogenic diet can lower glucose levels, increase β -OHB levels, and reduce tumor size or weight. Additionally, it can also impact brain tumor vascularity and gene expression and potentially influence survival time, making it a promising approach for managing brain cancer in humans. In conclusion, there is evidence showing that the Ketogenic diet has the potential to treat brain cancer. Due to the duration and sample volume of the previous studies, future research still needs to continue.

Keywords: Ketogenic Diet, Brain Cancer, Glioma, Ketone Bodies

1. Introduction

The ketogenic diet is a low-carbohydrate, high-fat, and adequate-protein diet, and it has been popular among people for its ability to treat type 2 diabetes and obesity, as well as for weight control.. In recent decades, more functions of KD have been determined, such as the treatment of cancer. Despite a relatively low incidence rate compared to other cancers, such as lung and breast cancer, brain cancer has a high mortality rate. According to the National Cancer Institute, the 5-year mortality rate of brain and other nervous system cancer was about 66% from 2013 to 2019 [1]. Apart from that, the traditional treatments for brain cancer are radiotherapy or chemotherapy, which are invasive and have limited efficacy. Therefore, the ketogenic diet can treat or prevent glioma, which is one of the main brain cancer types with a high malignancy risk like other types of brain cancer. However, the ketogenic diet for the treatment of glioma is a novel therapy that has not yet been systematically applied on a large scale in clinical settings. As a result, this paper discusses the treatments of KD for brain glioma and its limitations by reviewing seven previous papers.

2. Methods

This paper conducted a literature research using subject headings such as “ketogenic diet” and “brain cancer” or “ketone bodies” and “glioma” to identify relevant studies. The inclusion criteria for studies included animals injected with tumor cells or patients diagnosed with glioma, which were fed with a ketogenic diet for at least ten days. The reviews and non-glioma brain cancer research were excluded.

The single-group experimental studies of mice and two case studies are included in this review. In some of the mouse studies, the tumor was implanted into the cerebral cortex of mice. Then, the relative metabolic index was measured, like the glucose level, weight changes of the mouse body, the tumor weight, insulin growth factor 1 (IGF-1), cholesterol, and lipid levels. According to Figure 1, 54 papers were screened, and seven papers will be discussed in this review through selection. Meanwhile, the table of study characteristics is listed below in table 1.

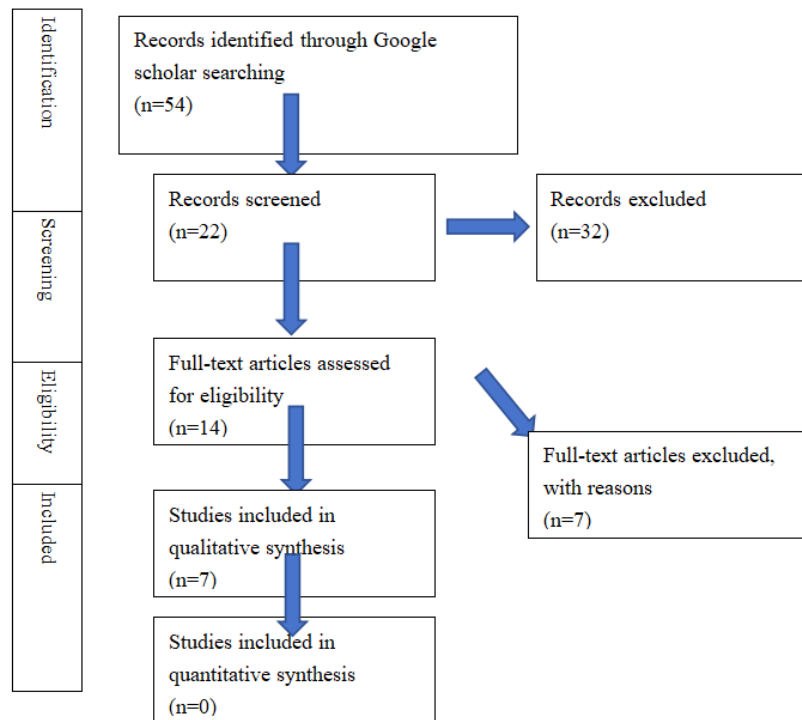


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses Chart

Table 1. Study Characteristics

Reference/Year	Design	Samples	Age range	Intervention	Duration	Outcomes
Seyfried et al. (2003) [2]	Single-arm pilot trial	33 (6 KD-R; 14 KD-UR)	10 - 12 weeks old male mice	SD-UR, SD-R, KD-UR, KD-R	13 days	Energy intake, body weight, tumor dry weight, plasma glucose, beta-HB, IGF-1
Zhou et al. (2007) [3]	Single-arm pilot trial	36-42 (12-14 mice per group)	10 - 12 weeks old male mice; human	SD-UR, KC-UR, KC-R	11 days	Body weight, Glucose level, Beta-OHB, Tumor wet weight, SCOT and beta-OHBDH expression

Table 1. (continued)

Woolf et al. (2015) [4]	Single-arm pilot trial	11 (6 KC)	10 week old female mice	SD, KC	21 days	Beta-HB, hypoxic response, tumor microvasculature, edema, gene expression, junction protein and aquaporins; body weight
Abdelwahab et al. (2016) [5]	Single-arm pilot trial	11 KC	10 weeks mice	SD, KC	150-200 days	cohort size, percentage of survival, Blood ketone, glucose levels, Animal weights
Stafford et al. (2010) [6]	Single-arm pilot trial	20	female C57BL/6 mice	KD, SD	Two weeks to 36 days	β -hydroxybutyrate levels, blood glucose level, ROS; body weight; percentage of survival
Schwartz et al. (2015) [7]	Case study	7	Patients with advanced brain tumors (age: 56; 5.5)	KD	12 weeks; 5 years	Blood glucose, ketones, and daily weights.
Perez et al. (2010) [8]	Case study	5	Pales, n = 3; median age 4.4 years; range, 2.5-15 years	KD	9 months to 30 months	Glucose level, Ketone bodies, body weight, serum lipid levels, the disorders of taking KD diet

3. Results

3.1. Glucose Level, β -OHB Level, and Their Effects on Tumor

All the studies assessed the glucose level, or the β -OHB level. They all found that glucose levels decreased and/or the β -OHB level increased significantly with the KD implied. Only one patient from a case study showed the KD related high ketosis of 7.2 mmol/L in the blood (hyperketatosis defined as >6 mmol/L) and one borderline glucose level of 2.4 mmol/L (hypoglycemia defined as <2.5 mmol/L) on the 26 and 3 days after adapting the KD diet, but the two indexes returned to normal range in the other days. Two studies showed that consuming the KD ad libitum does not induce substantial modifications in plasma glucose levels. However, the restricted group's glucose level was significantly lower than the unrestricted one. Three studies reported that the β -OHB level increased in the KD or energy-restricted groups but showed no relationship with the glucose level. Additionally, the restricted

dietary conditions in mice result in significantly higher circulating β -OHB levels than unrestricted conditions.

3.2. *Body Weight*

All seven studies reported body weight changes with time, with five illustrating a faster weight decrease with time for the KD group than the standard diet. One case study showed no change in the five patients' body weight, and one mice study showed no significant difference between SD and KD diet. One study found that the unrestricted dietary pattern has no difference between KD and the standard diet, and the restricted were more physically active than the unrestricted.

3.3. *Tumor Size or Weight*

Six studies reported tumor size or weight decreasing in the KD group, which is highly related to survival length, according to Stafford et al. [6]. One study found that the restricted group decreased more significantly than the unrestricted group. Tumor dry weight was 86% lower in the SD-R group compared to the SD-UR group and 80% lower in the KD-R group than the KD-UR group.

3.4. *Oxygen Species in Tumor Cells*

One study showed increased ROS in the SD group in vivo analysis, while ex vivo analysis revealed a significant reduction in ROS levels in the KD group. Some tumor cells displayed higher ROS levels, indicating potential resistance.

3.5. *Brain Tumor Vascularity*

Three studies reported the effects of KD on brain tumor vascularity. One study used Factor VIII immunostaining to examine blood vessel densities in brain tumors. The results showed fewer blood vessels in the restricted KD group, and no significant differences were found in the unrestricted KD and SD groups. Another study found that the KD significantly decreased VEGFR2 protein expression.

3.6. *Gene Expression*

One study found that mice with the KD diet had 1129 genes differentially expressed in tumors compared to normal brains, while SD animals had 1015 differentially expressed genes (614 genes were commonly differentially expressed in both groups). According to ANOVA analysis, the KD significantly impacted gene expression, causing a shift towards a pattern closer to that observed in normal brains. Woolf et al. [4] also determined that the KD can alter the expression of carbonic anhydrase IX and proteins involved with the hypoxic response: Tumors from mice fed a KC exhibited notable decreases in both CA IX and Hypoxia-inducible factor-1 α (HIF-1 α) levels compared to those fed a SD. Additionally, a significant reduction in VEGFR2 expression was observed in the KC group.

3.7. *Survival Time*

Four studies reported the KD effects on the survival of samples. The results illustrated that the restricted KD could extend survival time in mice with metastatic cancer, while the difference was insignificant for the unrestricted KD compared to SD. One of the four papers also found that one mouse sample treated with KD appeared to be cured of its tumor, and no evidence showed the tumor regrowth for an additional 200 days.

4. **Discussion**

The ketogenic diet shows the potential to decrease the glucose level, increase β -OHB level, and lower the tumor size or weight. Additionally, it can control body weight and affect survival time, which has the potential to be a practical approach for managing brain cancer in humans. Stafford et al. [6] found the KD diet can reduce the ROS level, which has dual effects on tumorigenesis and tumor heterogeneity. They can act as signaling molecules that promote tumor growth. Still, high levels of ROS can also be

detrimental to cancer cells, causing genotoxicity and potentially inducing apoptosis, according to de Sá Junior [9].

Additionally, the reduction of CA IX HIF-1 α levels and VEGFR2 was found in KC group. HIF-1 α and CA IX are hypoxia markers, and the expression of CA IX is also controlled by HIF-1 α . The overexpression of CA IX in malignant glioma is strongly associated with poor patient survival by Proescholdt et al. [10]. VEGFR2 is the main regulator of tumor angiogenesis, inhibiting it can produce radiologic response, normalize tumor vasculature, reduce edema, and improve patient quality of life by Jain [11]. Therefore, the restricted KD more efficiently affects brain tumor vascularity and adjusts the expression of several proteins involved in malignant progression. Accordingly, the ketogenic diet can be used as adjuvant therapy combined with radiation or chemotherapy by Seyfried et al. [2], reducing the invasive harm of the traditional treatment.

However, individual patient dietary preferences limit the feasibility of implementing uniform ketogenic diet regimens in clinical trials. Perez et al. [8] found some side effects of adapting KD in their case study. Vomiting, food refusal, fatigue, headache, and inability to swallow occurred in the five patient samples. Furthermore, the small sample sizes and lack of proper randomization and control groups in most human studies make it difficult to draw conclusive and generalizable findings regarding the effects of the ketogenic diet on cancer growth and survival in humans. Plenty of studies still need to be done to confirm the feasibility of clinical practice.

5. Conclusion

In conclusion, the ketogenic diet can potentially treat glioma by decreasing the metabolism process, gene expression, and oxygen species in tumor cells, reducing the tumor size, and increasing the survival time summarized from these seven studies. However, some side effects also appear in some clinical samples, and some syndromes have been mentioned above. The experimental duration is also limited, usually from a few days to a few months. Additionally, whether the ketogenic diet has been reversed, whether it has had a positive effect on the treatment of cancer, or whether it has had a detrimental effect in other areas is unknown, or there is very little research data available.

Anyway, the therapy of KD on glioma is still a new field of treating glioma. As a standalone treatment or as an adjuvant therapy alongside radiation or chemotherapy, it can potentially improve clinical outcomes and enhance the quality of life for brain tumor patients, reducing the invasive harm the traditional methods cause. Accordingly, it can be combined with traditional therapy to enhance the overall efficacy of brain cancer treatment. The exact mechanism also needs to be determined in future studies, which can help identify novel therapeutic targets and develop more targeted approaches. Therefore, further long-term effects, safety studies, and clinical practice are needed to test its safety and feasibility.

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The reasons and solutions for lipso-trichia and pelada phenomenon

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Abstract. According to Darwin's evolution, people know that humans evolved from certain types of apes which used to have a lot of hair. In the process of evolving from apes to humans, apes' hair was lost gradually. Nowadays, there is only a little bit of hair that modern humans still left, like hair on the heads and four limbs. NLM database reported an experiment that justified hair on the heads can protect human brains by reducing solar radiation. But many people, because of some reasons, like genes, immune system, and endocrine, have hair loss problems, which leads to possible damage to their brain and appearance. This paper aims to explore the way to mitigate scarring alopecia and androgenetic alopecia. The results show that people can prevent their hair by changing their life habits cleaning their scalps carefully and having a good mood. If they already get scarring alopecia, people must take medicine based on medical advice. And if people get androgenetic alopecia, they should use Minoxidil. This paper can help people with hair loss problems grow their hair back so that it can better help the brain dissipate heat.

Keywords: Hair, lipso-trichia, pelada, prevent, treat.

1. Introduction

Nowadays, an increasing number of people lose their hair and even many young people seek help online on how to get more hair. As for the causes of hair loss in old people, a lot of seniors are at risk of hair loss because as they age, the hair matrix cells which are in hair follicles degenerate gradually, and therefore less hair protein is produced. And 4 main factors contribute to hair loss in young people. Firstly, people in stressful environments and sleeping badly can lead to hair loss. It is because chronic stress and poor sleep can lead to endocrine imbalance and thus hair loss. Eating poorly can also cause hair loss because fewer nutrients are provided to the hair matrix cells. Finally, some medical treatments may lead to hair loss, such as radiotherapy which can hurt hair follicles. Tina Lasis, James W. Snallcombe, and a lot of people experimented. They utilized warm puppet and human hair hairpieces at various breeze speeds in a temperature and moistness-controlled climate, with and without reenacted sun-powered radiation, and they gathered information on the convective radiative, and evaporative intensity transitions to and from the scalp about properties of the scope of hair morphologies, as well as a bare scalp. They proved that hair plays an important role in dissipating. So it is necessary to treat lipso-trichia and pelada. This paper, by analyzing the available literature and data, mainly pays attention to the 2 types of hair loss (scarring alopecia and androgenetic alopecia) and how to prevent and treat them so that human hair can help the brain reduce solar radiation better. For the 2 types of hair loss, there is the

same method to prevent them, and that is to get healthier life habits, such as getting up and going to bed early. And there are different ways to treat them respectively, like reducing some food and getting medicines. This paper will introduce the 2 types of hair loss and provide the methods to treat them.

2. Three types of hair loss

2.1. Androgenetic alopecia

Androgenetic alopecia is the most well-known type of baldness in people and is a typical physiologic variation. It is most pervasive in white men, with 30%, 40%, and half encountering androgenetic alopecia at 30, 40, and 50 years old, separately. Albeit this condition is more uncommon in ladies, 38% of ladies more seasoned than 70 years might be impacted. Numerous patients with androgenetic alopecia have a family background of this condition. Hair diminishing happens in a sex-explicit example. Men commonly present with bitemporal diminishing, diminishing of the front-facing and vertex scalp, or complete balding with lingering hair at the occiput and transient fringes.⁵ Ladies regularly present with diffuse hair diminishing of the vertex with saving of the front-facing hairline. A few ladies experience diminishing over the horizontal scalp. Normal circumstances that mirror androgenetic alopecia incorporate thyroid sickness, iron inadequacy weakness, and hunger [1].

2.2. Scarring alopecia



Figure 1. Patchy Baldness Characteristics [2].

Scarring alopecias are a gathering of immovable and unprecedented balding problems described by long-lasting hair follicle obliteration. The most ordinary clinical sign of cicatricial alopecia is the deficiency of noticeable follicular ostia in a scarring region(a). The histopathological sign of a completely evolved injury is the substitution of the hair follicle structure for stringy tissue. Cicatricial alopecia might result from injury (consumes, radiation, footing), infiltrative cycles (sarcoidosis, carcinomas), or contamination (dermatophyte) In those circumstances, the hair follicle is a “by-stander” sadly engaged with more worldwide harm in the scalp; consequently, long-lasting balding is an optional occasion (optional cicatricial alopecia). In contrast, essential cicatricial alopecias (PCA) are a gathering of problems, where the hair follicle is the fundamental objective of damaging irritation bringing about irreversible balding. PCA incorporates the states of different clinical and obsessive elements. This, along with conflicting utilization of phrasing, has hampered a far-reaching meaning of the clinicopathological relationship, which has made the investigation of PCA pathophysiology troublesome. A leap forward in how we might interpret PCA was made when hair follicle undeveloped cells were recognized in the

lump region of the hair follicle. Since the irritation in PCA generally includes the lump locale, it is currently broadly acknowledged that the deficiency of hair follicle undifferentiated cells is the primary justification for super-durable alopecia. Right now, PCA draws significant interest from clinicians and foundational microorganism researchers as a model of organ-explicit undeveloped cell exhaustion. In the current paper, late advances in getting it and the board of PCA are evaluated, particularly zeroing in on current bits of knowledge into etiopathogenesis. Point-by-point portrayals of the clinical and neurotic elements of individual PCA are out of the extent of this survey [2]. Scarring alopecia causes long-lasting balding from the obliteration of the hair follicles by fiery or immune system infections. The most widely recognized reason for this is discoid lupus erythematosus, which produces decayed erythematous patches, in some cases with telangiectasia [3].

3. The prevention of hair loss

If people don't want to lose their hair, they must take some precautions. Firstly, they need to get less food that enriches with Vitamin D and more food that enriches Vitamin B, C, and iron. It is because excess Vitamin D can cause calcium to be deposited on the surface of the scalp, blocking hair follicles and leading to hair loss; vitamin B can reduce sebum production in the scalp and reduce the irritation of hair follicles due to scalp inflammation; Vitamin C can help scalp with antioxidants; and iron can increase the number of keratin which is an important component of hair. Besides, early rising, early night (avoid overwork), and avoiding prolonged stress are good for endocrine balance. People should avoid over-styling, which means using fewer hair dryers and hair curling irons (heat may hurt hair). Regular scalp cleaning is also necessary. It can reduce scalp sebum [3].

4. The methods to treat hair loss

4.1. For androgenetic alopecia

The pathophysiology of androgenetic alopecia is related to the action of androgens in the scalp follicles. The action of these androgens may be related to circulating levels of the hormones as well as to genetic predispositions that influence the activity of these androgens in the periphery. The evolutionary classification of alopecia will follow Norwood for men and Ludwig for women. Hormonal testing will be performed in women only in the context of research or identification of hyperandrogenism; this is essential to guide treatment. Diagnosis and treatment follow-up are inherently clinical but can be aided by three diagrams that will show the miniaturization process of androgenetic alopecia and resting alopecia in affected areas. Treatment of hair loss can be localized or systemic. Topical treatment for both males and females involves the use of 2% to 5% minoxidil lotion. For men, the 5 alpha-reductase 2 inhibitor finasteride has shown efficacy at a dose of 1 mg per day. For women, in the absence of a counterproposal, an estrogenic progestin with an antiandrogen will be used: cyproterone acetate or spironolactone. Therapeutic assessment will be made after 6 months of treatment. Topical treatment for both males and females will be 2% to 5% minoxidil lotion. In men, the 5 alpha-reductase 2 inhibitor finasteride has shown efficacy at a dose of 1 mg per day. For women, in the absence of a counterproposal, an estrogenic progestin with an antiandrogen will be used: cyproterone acetate or spironolactone. Therapeutic assessment will be made after 6 months of treatment. Topical treatment for both men and women will be 2% to 5% minoxidil lotion. In men, the 5 alpha-reductase 2 inhibitor finasteride has shown efficacy at a dose of 1 mg per day. For women, in the absence of a counterproposal, an estrogenic progestin with an antiandrogen will be used: cyproterone acetate or spironolactone. A therapeutic evaluation will be performed after 6 months of treatment [4].

4.2. Other methods for androgenetic alopecia

Vitamin D is complicatedly associated with different flagging pathways of development and separation of hair follicles. Most examinations show a backward connection between serum vitamin D levels and non-scarring alopecias, for example, telogen exhaust, androgenetic alopecia, alopecia areata, and trichotillomania. Lack of vitamin D is likewise connected with scarring alopecia. Be that as it may,

convincing examinations show the advantage of vitamin D organization in adjusting to going bald and it is missing to deal with these circumstances. Consequently, further examinations are required before vitamin D can be regularly suggested as a treatment methodology in these circumstances [5].

4.3. *For scarring alopecia*

Because of the uncommonness of cases, researchers and specialists use techniques for Transplantation and oral Minoxidil to treat scarring alopecia. Alopecia is a typical concern experienced in clinical practice. The treatment approach differs as indicated by the sort and seriousness of alopecia. Be that as it may, accessible treatment choices have restricted viability and a few unfavorable impacts. As of now, unique treatment choices are being contemplated to conquer these impediments. Moreover, cell pathways engaged with the pathophysiology of alopecia are further explained to target pathogenic particles possibly. What's in store looks extremely encouraging and new powerful medicines, for example, Janus kinase inhibitors could be accessible for alopecia areata. Undifferentiated cell innovation is progressing and organizations associated with hair follicle neogenesis are beginning clinical preliminaries on patients with androgenetic alopecia [6].

5. Conclusion

This paper introduces 2 types of lipotrichia and pelada and some solutions. Through analyzing a lot of documentation, people can find androgenetic alopecia is the most common form of hair loss and also a normal physiologic variant. And it is prevalent in white men and women who are older than 70. Scarring alopecia is a rare disease that is characterized by permanent hair follicle destruction. Now people think the main reason for permanent hair loss is the absence of hair follicle stem cells. To treat the 2 types of hair loss, people can use Minoxidil based on doctors' advice. Minoxidil plays a crucial role in treating alopecia. In the future, doctors and scientists should focus on the treatment of scarring alopecia and create new methods, without side effects, to treat it.

This paper summarises and analyses a large amount of existing literature and extracts some useful information on prevention and treatment to help people protect their hair and thus their brain.

Besides, this paper did not do any experiments as well as clinical investigations, so the particular reactions of patients to the drug are not clear, such as allergies, nor is it clear how long the drug has been used in different people (men, women kids, seniors).

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Research progress on treatments for Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is an irreversible neurological degenerative disease characterized by progressive cognitive impairment, and its pathogenesis remains unclear. Its main pathological features include neurofibrillary tangles formed by hyperphosphorylated tau protein and amyloid beta ($A\beta$) plaque deposition. As the disease progresses, the patient's self-care ability will become worse and worse. In the later stages, the patient will have to rely on others for daily life, which places a great burden on the patient, family, and society. Currently, there are a limited number of AD treatment drugs and their efficacy is limited, and they cannot effectively improve the condition of AD. This article reviews different methods for treating AD and provides a reference for the treatment of AD. At present, the treatment of senile dementia is mainly divided into two categories. The current treatment methods include Lencamab, bee therapy related drugs, NSCs transplantation therapy, Panax notoginseng saponins, and hyperbaric oxygen therapy. It is necessary to explore new treatment methods and drugs to improve treatment effectiveness and widely apply dementia in the elderly.

Keywords: Alzheimer's Disease, Beta-amyloid, Mechanism of Action, Therapeutic Effect, Function

1. Introduction

Alzheimer's disease is a common neurodegenerative disease that seriously affects cognitive function and quality of life in patients. The treatment methods for Alzheimer's disease are still limited, and currently, new treatment methods such as lencamizumab, bee therapy related drugs, NSCs transplantation, and total saponins of Panax notoginseng have attracted much attention.

However, in clinical applications, there are still some issues that need to be addressed with these treatment methods. Therefore, the purpose of this literature review is to explore the efficacy and safety of these new therapies in the treatment of Alzheimer's disease, as well as their impact on cognitive function and quality of life in patients. By studying these therapies, better treatment outcomes and improved quality of life for patients can be achieved, while providing new ideas and methods for the treatment of Alzheimer's disease. Therefore, research on these drugs is of great theoretical significance and practical value.

2. Treatment of lencanezumab in early Alzheimer's disease

2.1. Basic Introduction

Lencanezumab, as a new drug for the early diagnosis of Alzheimer's disease (AD), mainly targets mild cognitive impairment and mild dementia. Its mechanism of action is to target soluble $A\beta$ and also has

an inhibitory effect on insoluble fibrils [1, 2]. The pathological process of A β is closely related to the inflammatory response. Activated astrocytes can efficiently phagocytose damaged cells, damaged synapses, and proteins, and play a key role in maintaining brain homeostasis [3]. During the development of AD, astrocytes excessively uptake A β 42, causing it to accumulate intracellularly, causing damage to endosomes and lysosomes, and releasing toxic extracellular vesicles, damaging neurons [3]. Lencanezumab is an A β -specific antibody that can penetrate the blood-brain barrier in vivo, bind to A β 42, promote its removal of pathological A β , reduce A β deposition, and thus protect neurons from A β damage [3].

2.2. Clinical trials and effects

Currently, lencanezumab has been evaluated in three phases of clinical trials. The results found that lencanezumab at 10 mg/kg every 2 weeks significantly slowed the decline in cognitive function at 18 months compared with placebo and showed benefits at 6 months that lasted until 18 months. The degree of cognitive function decline was reduced by 25% [4]. In addition, trials have shown that lencanezumab is effective, safe, and tolerable in the treatment of early-stage AD. Patients with early-stage AD have significant improvements in cognition, function, and behavior.

However, due to certain limitations in the convenience and popularity of intravenous injection treatment, although lencanezumab has been approved for the treatment of early AD, its limitations and adverse reactions still need to be overcome before it can be widely used. Application [1]. Clinical trials are still ongoing to fully evaluate its efficacy and safety, especially in late-stage Alzheimer's disease.

3. Apitherapy related drugs to treat Alzheimer's disease

3.1. Basic introduction and mechanism of action

Apitherapy is a method of using bee products as medicine to treat diseases, including honey, bee pollen, royal jelly, propolis, bee venom and other bee natural products [5], which are rich in active substances that are considered to have the potential to treat neurodegenerative diseases [5-7]. In addition, wasp venom (WV) contains a variety of neuroactive molecules, which may provide new ideas for studying the physiological functions of the nervous system and the pathological changes of related diseases [8].

The mechanism of action of apitherapy-related drugs on Alzheimer's disease is mainly reflected in the following aspects: First, the active ingredients in apitherapy drugs may help inhibit the accumulation of beta amyloid (A β) in the brains of patients with Alzheimer's disease. Their toxicity is reduced; secondly, these drugs may combat neurodegeneration by regulating the physiological functions of the nervous system [9]. In particular, bee venom, which contains a variety of neuroactive molecules, may provide a new research idea for studying neurological functions and related diseases [8].

3.2. Treatment effect

Research shows that apitherapy drugs such as honey, royal jelly, propolis, and bee venom are rich in active ingredients and have the potential to treat neurodegenerative diseases. In particular, honey, as a common food, has the advantages of being easy to obtain, economical and cheaper than existing clinical drugs for Alzheimer's disease [9].

However, although preliminary results show the therapeutic potential of apitherapy-related drugs, there are still some issues that require further study, including the optimal clinical concentration and pharmacokinetics of the active ingredients, as well as the exact inhibitory effect on A β aggregation, and the improvement of symptoms of toxicity. and binding site mechanisms. Therefore, as a new method for the treatment of Alzheimer's disease, apitherapy-related drugs require more scientific experiments and clinical verification to determine their safety and effectiveness [9].

4. Neural stem cell transplantation to treat Alzheimer's disease

4.1. Mechanism of action

The mechanism and therapeutic effect of neural stem cell transplantation therapy for Alzheimer's disease (AD) have attracted much attention in recent research. Studies have pointed out that the intervention effect of transplanted neural stem cells (NSCs) on AD is multifaceted. First, NSCs can successfully differentiate into damaged neurons with functional neurons and establish synaptic connections with surrounding remaining neurons, which is expected to play a role in disease treatment. Secondly, transplanted human neural stem cells (HNSCs) can effectively reduce the phosphorylation level of Tau protein in the lateral ventricles of mice, although the mechanism is unclear [10].

Research also shows that as the disease progresses, transplanted NSCs release neurotrophic factors, which are expected to increase synaptic density and reverse cognitive impairment. These neurotrophic factors may prevent additional cognitive decline, and their impact on hippocampal nerves also deserves further study [11]. Although AD animal models are not yet sufficient to accurately simulate human AD, intervention from the preclinical stage may help inhibit the progression of AD [10].

4.2. Treatment effect

NSCs transplantation therapy for AD, which is considered to have great potential, may play a role in multiple fields. First, it may have a protective effect on microvessels and reduce the deposition of β -amyloid and TAU proteins. Secondly, NSCs transplantation may also relieve inflammatory reactions in the brain, especially chronic inflammatory reactions [10], which are caused by aging. In addition, studies have also pointed out that NSCs transplantation may have anti-inflammatory effects [12,10] and can help prevent the progression of AD.

In general, the potential therapeutic effects of NSCs transplantation include protecting blood vessels, reducing abnormal protein deposition, alleviating inflammatory reactions, and anti-inflammatory effects. However, further research and development are still essential, especially more complete studies on animal or cell models [10], because clinical trials have not yet produced positive results.

5. Panax notoginseng total saponins treat Alzheimer's disease

5.1. Basic introduction and mechanism of action

Panax Notoginseng Saponins (PNS) is the main active ingredient of Panax notoginseng, a plant of the Araliaceae family, and is extracted from the dried roots of Panax notoginseng. Commonly used clinically for cardiovascular and cerebrovascular diseases. Its main active ingredients include ginsenosides Rb1, Rg1, Rd, Re, notoginseng saponin R1, etc., these ingredients account for more than 90% of the total saponins. These components endow Panax notoginseng total saponins with various biological functions such as immunomodulation, anti-tumor and so on [13].

PNS may exert pharmacological effects through its antioxidant function, and inhibiting the MTOR signaling pathway is one of its core targets. In PC12 cells, PNS improved the autophagy of AD-like nerve damage induced by A β 25–35 by inhibiting the activation of the MTOR pathway [14]. PNS has also been found to improve brain perfusion blood flow, reduce inflammation levels, and enhance patients' cognitive function in AD patients, showing good clinical application value [15].

5.2. Therapeutic effect

In the treatment of Alzheimer's disease, Panax notoginseng saponins have shown certain effects. Studies have shown that Panax notoginseng saponins play a key role in improving cerebral blood perfusion and reducing inflammation in patients with Alzheimer's disease. By regulating blood flow and improving brain perfusion, Panax notoginseng saponins help relieve symptoms of neurological disorders in patients with Alzheimer's disease [15].

In the treatment of Alzheimer's disease, the anti-inflammatory effect of Panax notoginseng saponins also plays a certain positive role. Inflammation plays a crucial role in the pathogenesis of Alzheimer's

disease, and the anti-inflammatory effect of *Panax notoginseng* saponins can help patients reduce inflammation levels, thereby slowing down the progression of the disease [15].

In addition, in the treatment of Alzheimer's disease, the improvement of cognitive function by *Panax notoginseng* saponins is also one of its key contributions. Studies have found that *notoginseng* saponins can improve patients' cognitive function to a certain extent and help improve patients' quality of life [13,15].

6. Hyperbaric oxygen therapy for Alzheimer's disease

6.1. Mechanism of action

Multiple mechanisms may be involved in the improvement of learning and memory, spatial exploration, and cognitive function in Alzheimer's disease (AD) rats treated with hyperbaric oxygen therapy. Firstly, hyperbaric oxygen therapy may combat cerebrovascular and neurological diseases, by increasing cerebral blood oxygen content, enhancing neuronal metabolism, and improving cerebral blood flow [16]. Secondly, hyperbaric oxygen therapy may promote the survival and functional maintenance of neurons, protecting them from damage by alleviating inflammatory reactions and oxidative stress [17]. In addition, hyperbaric oxygen therapy may also enhance nerve resistance against neurodegenerative diseases by promoting the formation of synapses in the nerves. Research has shown that the recovery of hippocampal function in rats with hyperbaric oxygen therapy may be related to the increased insulin sensitivity that further affects normal neuronal function [18,19].

6.2. Clinical trials and treatment effects

In the experiment, hyperbaric oxygen therapy showed positive effects on learning, memory, spatial ability, cognitive function, and other aspects of Alzheimer's disease (AD) rats. The study used animal experiments by placing rats in the hyperbaric oxygen treatment group, using 100% pure oxygen 2ATA daily for 1 hour each time, and slowly pressurizing and decompressing. The experimental results showed that the rats in the AD model group showed significant improvement in spatial exploration experiments after hyperbaric oxygen treatment. The number of times the treatment group's platform has significantly increased, the number of times it passes through the target quadrant has significantly increased, the time it stays in the target quadrant has significantly increased, and the time it takes to reach the platform for the first time has significantly decreased [18].

In addition, hyperbaric oxygen therapy significantly restored insulin sensitivity, hippocampal function, and cognitive ability in aging rats, indicating that this treatment method has the potential for improvement in neurological and neurodegenerative diseases with mild cognitive impairment, and even cognitive function improvement, brain metabolism, etc. [19]. It is worth noting that compared with the AD model group, the treatment group showed a significant decrease in avoidance latency in the localization navigation experiment, showing a significant difference.

The potential therapeutic effect of hyperbaric oxygen therapy on senile dementia is demonstrated by improving the learning, memory, exploration ability, and cognitive function of AD rats. This study provides experimental evidence for the application of hyperbaric oxygen therapy in neurological diseases, providing useful inspiration for future in-depth research and application.

7. Current treatment methods and future trends

At present, the treatment of senile dementia is mainly divided into two categories. The current treatment methods include Lencamab, bee therapy related drugs, NSCs transplantation therapy, *Panax notoginseng* saponins, and hyperbaric oxygen therapy. These treatment methods mainly manifest as the protection of the nervous system against inflammation of cognitive function, and some therapeutic effects and potential have been demonstrated in clinical trials. However, these benefits still need further in-depth investigation. The future research direction should include discovering more therapeutic drugs, not limited to Western medicine or traditional Chinese medicine, but should combine Chinese and Western medicine to achieve more significant therapeutic effects. Further research will be conducted on these

treatment methods, particularly in the validation of animal models and clinical trials. At the same time, it is necessary to explore new treatment methods and drugs to improve treatment effectiveness and widely apply dementia in the elderly. The comprehensive use of multiple treatment methods may provide more effective treatment options for people with Alzheimer's disease.

8. Conclusion

This article reviews the research progress of treatment methods for Alzheimer's disease, mainly including lencamab, bee therapy related drugs, neural stem cell transplantation, total saponins of *Panax notoginseng* and hyperbaric oxygen therapy. Lunkanaizumab targets A through targeted action β Solubility of A alleviates A β Deposition of A protects neurons from A β Damage caused by. The active substances in bee therapy related drugs and the neuroactive molecules in bee venom, which are considered to have the potential to treat neurodegenerative diseases, also provide new ideas for studying neurological diseases. The mechanism of neural stem cell transplantation for Alzheimer's disease includes differentiation of competent neurons, repair of damaged neurons, and a decrease in Tau protein phosphorylation levels. As the main active ingredient of *Panax notoginseng*, its total saponins have various biological functions, such as immune regulation and anti-tumor effects. The potential therapeutic effect of hyperbaric oxygen therapy on senile dementia is demonstrated by improving the learning, memory, exploration ability, and cognitive function of AD rats.

These studies provide more treatment options for Alzheimer's patients and offer new ideas and approaches for the treatment of this disease. Each treatment method has its advantages and disadvantages. With the development of science and technology, more effective and convenient treatment methods will inevitably emerge.

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Research progress on the role of β -glucan in obesity suppression through the gut microbiota

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Abstract. Globally, the prevalence of chronic illnesses is on an upward trajectory. With the number of patients suffering from hypertension, cardiovascular diseases, or obesity rising globally, world's population will be severely affected in their daily lives and work. Metabolic dysregulation caused by obesity can likely lead to a series of metabolic diseases such as hypertension, hyperlipidemia, and diabetes, while also increasing the risk of neurological disorders such as cerebral infarction or hemorrhage. Numerous studies have shown that regulating the gut microbiota through the consumption of β -glucan can effectively address obesity from multiple perspectives. Therefore, researching the mechanism by which β -glucan affects chronic diseases through its action on the gut microbiota holds significant implications for the prevention and treatment of chronic diseases.

Keywords: β -glucan, Gut Microbiota, Obesity

1. Introduction

The incidence of chronic diseases worldwide is increasingly high, with the number of patients suffering from hypertension, cardiovascular diseases, or obesity rising globally. The “World Obesity Federation” predicts that by 2035, over 4 billion people will be categorized as obese or overweight, indicating that half of the world's population will be severely affected in their daily lives and work. The gut microbiota significantly contributes to the prevention and management of obesity, showcasing its beneficial impact through its evolutionary development alongside humans. This evolution has empowered it to modulate the human immune system and play a crucial role in the onset of chronic illnesses. Recent research underscores a strong link between the alteration of gut microbiota's composition and functionality and the emergence of chronic conditions such as obesity, type II diabetes, and fatty liver disease. Maintaining a balanced gut microbiota is crucial for ensuring harmony between the intestinal ecosystem and its host. An imbalance or dysbiosis in the gut microbiota is increasingly recognized as a critical element in the genesis of metabolic disorders. This imbalance leads to metabolic disturbances and encourages the growth of disease-causing microbes. Therefore, researching the impact mechanisms and modes of action of the gut microbiota on chronic diseases is of great importance for the prevention and treatment of chronic diseases [1].

2. Literature Review

2.1. Progress in Obesity Research

The issue of obesity is escalating at an alarming rate worldwide, impacting approximately 107.7 million children and 603.7 million adults. This condition is a major contributor to mortality, with over 60% of deaths related to a high Body Mass Index (BMI). Should the current trajectory persist, projections indicate that by 2025, approximately 18% of males and 21% of females globally will be obese [2]. Moreover, obesity among adolescents is a significant health challenge, especially in affluent nations, where it affects more than 20% of the youth population. The incidence of extreme obesity in this demographic has seen a substantial increase, quadrupling since 1985. Adolescents facing obesity are at risk of several negative outcomes that affect their emotional well-being, social interactions, and physical health, including potential impacts on their growth and developmental paths. Most adolescents with obesity carry an increased risk of cardiovascular metabolic diseases and certain cancers into adulthood due to carrying excess fat [3]. In clinical trials, a formula combining weight and height is the most common and simplest estimate of body fat. In the realm of epidemiological research, the disparity in weight among individuals of the same stature is predominantly linked to body fat differences, with the Body Mass Index (BMI) serving as the primary measure for these variations. An overview of reliable body fat measurement techniques for clinical use is detailed in Table 1. Utilizing BMI to categorize levels of overweight and obesity illuminates the growth in body fat, enabling effective weight status comparisons both within groups and across different populations. Such comparisons are crucial for identifying at-risk demographics for health complications and premature death. Moreover, understanding BMI classifications aids in prioritizing health interventions on an individual and community basis and in gauging the success of these measures. It's important to recognize that due to differences in body proportions, BMI may not correspond to the same degree of obesity across different populations [3]. BMI is a crucial indicator for determining overweight and obesity.

Table 1. Defining Obesity

Method	Definition	Advantages/limitations
BMI	Weight in kilograms divided by square of the height in metres	BMI correlated strongly with densitometry measurements of fat mass; main limitation is that it does not distinguish fat mass from lean mass
Waist Circumference	Measured (in centimetres) at midpoint between lower border of ribs and upper border of the pelvis	Waist circumference and waist-to-hip ratio provide measures for assessing upper body fat deposition; neither provide precise estimates of intra-abdominal (visceral) fat
Skinfold Thickness	Measurement of skinfold thickness (in centimetres) with callipers provides a more precise assessment if taken at multiple sites	Measurements are subject to considerable variation between observers, require accurate callipers and do not provide any information on abdominal and intramuscular fat
Bioimpedance	Based on the principle that lean mass conducts current better than fat mass because it is primarily an electrolyte solution; measurement of resistance to a weak current (impedance) applied across extremities provides an estimate of body fat using an empirically derived equation	Devices are simple and practical but neither measure fat nor predict biological outcomes more accurately than simpler anthropometric measurements

2.2. The Biological Activity of β -glucan and Its Impact on the Gut Microbiota

β -Glucan, recognized as a valuable soluble dietary fiber, is derived from a diverse array of sources, including but not limited to, oats, barley, certain seaweeds, grains, as well as mushrooms and yeasts. Beyond these, it's also found within a range of bacteria and fungal species such as *Pneumocystis carinii*, *Cryptococcus neoformans*, *Candida albicans*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Histoplasma capsulatum*. Structurally, β -glucan comprises a complex chain of over 250,000 D-glucose units, intricately linked by β -glycosidic bonds, highlighting its natural polysaccharide identity. The structure of β -glucan varies depending on its source; for instance, the β -glucan found in mushrooms has short β (1,6) branches off a β (1,3) main chain, whereas the β -glucan from oats and barley consists of linear β (1,4) bonds with shorter β (1,3) structural chains (Figure.1) [4]. Foods rich in β -glucan are considered important candidates for healthy diets due to their multiple biological activities.

The gastrointestinal tract of higher animals harbors a large community of microorganisms (gut microbiota), including bacteria, archaea, viruses, and fungi. Scientists have discovered that the gut microbiota of higher animals is associated with various health-promoting activities. Modifications within the gut microbiota have profound impacts on both the physiological and functional states of the host organism. Foods containing prebiotics, aimed at bolstering gut microbiota populations, include high-fiber selections such as oats, mushrooms, seaweeds, and barley. Among these, β -glucan is identified as a pivotal prebiotic ingredient. Crucial for diabetes control are the stabilization of blood glucose, management of lipid levels, and regulation of blood pressure. Studies have demonstrated that dietary β -glucan intake effectively diminishes the challenges associated with diabetes, including its complications. Similarly, results suggest that *Ganoderma lucidum* (Reishi mushroom), rich in β -glucan, is used as an adjunct therapy for cancer treatment. Table 2 presents the effects of β -glucan from different sources on the gut microbiota and the health benefits generated [4].

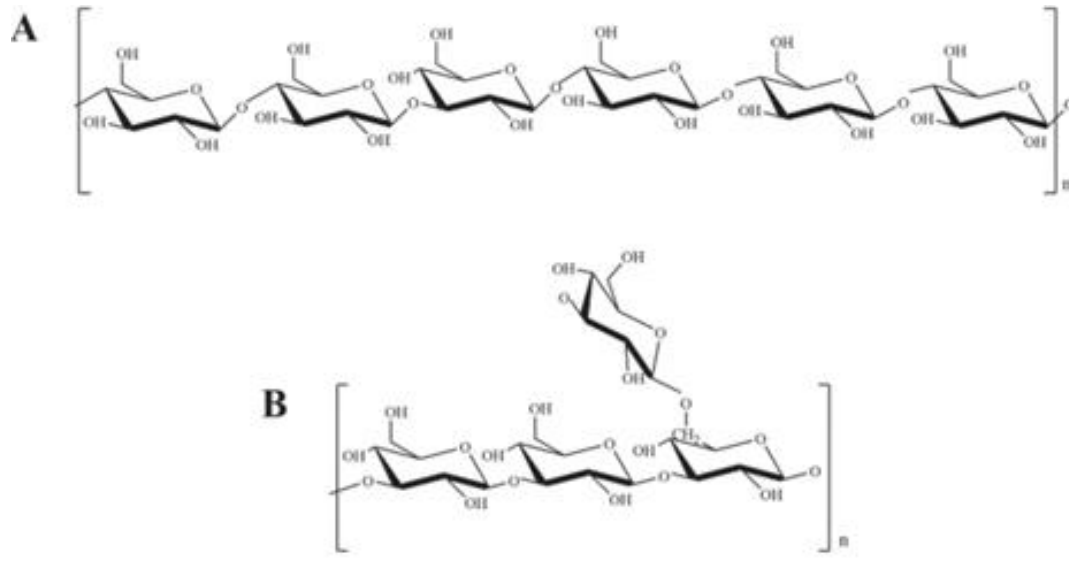


Figure 1. The structures of β -glucans [1]. (A) Cereals β -glucan and (b) Baker's yeast β -glucan

Table 2. Types of β -glucans, sources, regulation of gut microbiota and health benefits.

β -Glucans	Sources	Action on gut microbiota	Health benefits
Glomerellan	<i>Glomerella cingulata</i>	Glomerellan stimulates the immune system through microbiota.	Immune stimulant and anticancer property

Table 2. (continued)

LNT (Lentinan)	Lentinus(Lentinula edode)	Upon INT feeding, microbiota exhibit distinctly different space distribution. LNT reduced the diversity and evenness of gut microbiota	Synergistic action against breast cancer. Effective against colorectal cancer.
PSG(Polysaccharide Ganoderma)	Lucidum and Ganoderma atrum	Exact action on gut microbiota is not yetelucidated well	Exerts antitumor activity by activating mitochondria-mediated apoptotic pathway and boosting the immun systeml
Oats and barley beta glucans	Oats and barley	β -Glucans reduced the P-Cresyl Sulfate, LDL and total cholesterol levels through gut microbiota taxonomic composition modulation and changes in its metabolism.	Hypocholesterolemic activity
SR (Scleroglucan)	Sclerotium rolfsii or S. glucanicum	Exact role in microbiota is not elucidated well	Effective against colorectal cancer
PGG (betafectin)	Saccharomyces cerevisiae	The immunomodulation activity of PGG is through altering the microbiota population in gut	Immunomodulator property
Zymocel	Saccharomyces cerevisiae	No alterations on microbiota have been seen.	Shows in vivo immunopharmacological activity in mice
SPG (Sonifilan/schizophyllan)	Schizophyllum commune	The SCFAs produced from the action of microbiota on these prebiotics have the ability to prevent colon cancer.	Acts as a biological response modifier for mouse tumor systems. Protects against colon cancer
GRN (grifolan)	Grifola fondosa (maitake mushroom)	Butyrate is produced from this prebiotics via microbiota have many protective functions, such as differentiation regulator of mucosal gene expression and apoptosis	Grifolan from Grifola fondosa showed high antitumor activities

2.3. The Gut Microbiota and Obesity

During the 1980s, investigations identified a correlation between gut microbiota and obesity across human and rat populations, paving the way for further research. By the 1990s, advancements in molecular biology, particularly the use of 16S rRNA gene-based techniques, allowed for deeper insights into this association. The process by which gut bacteria ferment non-digestible polysaccharides to produce short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate, contributes significantly to the host's energy intake, ranging from 80 to 200 kcal/day. These SCFAs are essential for the host's energy supply and are absorbed by several organs. It has been observed that a reduction in dietary carbohydrates is linked to a decrease in both butyrate levels and the bacteria that produce it in obese individuals' stool samples. Further research corroborates that a low-fiber diet leads to a reduction in fecal butyrate, total SCFAs, and Bifidobacteria counts in obese patients. Thus, dietary fibers have a profound impact on the gut microbiota's composition and, consequently, on the host's overall health [5].

Metabolic dysregulation caused by obesity can likely lead to a series of metabolic diseases such as hypertension, hyperlipidemia, and diabetes, while also increasing the risk of neurological disorders such as cerebral infarction or hemorrhage. Over the past few decades, numerous scientists have found correlations between the gut microbiota, β -glucan, and the prevention and treatment of obesity-related diseases [1]. The gut microbiota has reached a mutualistic equilibrium with the human body over many years of coexistence. It participates in the host's energy metabolism, immune response, and biological antagonism. As a complex and dynamic system, it maintains the physiological balance of the body through long-term co-evolution with the organism and interaction with the environment [6]. In recent years, researchers have found that a wide range of diseases can be influenced by various types and degrees of changes in the gut microbiota, and now it has been discovered that regulating the gut microbiota with β -glucan can effectively address obesity. β -glucan promotes the production of butyrate by the human gut microbiota. If there is an enzyme in the intestine capable of degrading indigestible polysaccharides, then the number of fermentation end products (acetate and butyrate) in the feces of obese patients would increase, suggesting that the cause of obesity could be due to an increased energy intake. Some researchers have also found through mouse experiments that changes in the microbial population size and distribution of the gut microbiota are related to obesity. The gut microbiota, by engaging in various metabolic activities of the host, including the breakdown of diet, transformation of conjugated bile acids, and synthesis of certain vitamins and fermentation of indigestible dietary fibers to obtain energy from food, can also impact the body's metabolic activities [7].

3. The Mechanism of Anti-Obesity Action of β -glucan Through the Regulation of Gut Microbiota

3.1. Regulation of Gut Microbiota Composition by β -glucan

β -Glucan, a dietary polysaccharide extracted from grains, mushrooms, fungi, and bacteria, has been identified to play a crucial role in the modulation of gut microbiota, offering a therapeutic angle against obesity [7]. Research indicates a direct correlation between obesity and gut dysbiosis. Experimental data from obese mouse models show that β -glucan supplementation results in an altered gut microbiome, characterized by an increase in Bacteroidetes and anaerobes, and a decrease in lactobacilli and Enterobacteriaceae. Such dietary interventions lead to an augmented presence of Bifidobacteria and lactobacilli, escalating the production of short-chain fatty acids (SCFAs). These metabolic by-products activate immune responses, curbing inflammation linked to obesity [8]. Further observations reveal that β -glucan's impact on the gut flora—promoting beneficial bacteria while suppressing detrimental ones—is more pronounced with β -glucans of lower molecular weight, attributed to their superior fermentation and degradation capabilities in the gut.

3.2. The Fermentation Products of β -glucan Suppress Obesity

Through the intestinal microbiota's fermentation of β -glucan, short-chain fatty acids (SCFAs) are produced, which are instrumental in triggering a variety of gut hormones. These hormones are pivotal in enhancing carbohydrate digestion, lowering blood sugar, managing appetite, and modifying both the composition and metabolic activity of the gut microbiota, thus aiding in obesity reduction [9]. Experiments on live models have illustrated that β -glucan, particularly from oats, acts as a prebiotic that reaches the large intestine for fermentation into SCFAs. This is mirrored in vitro studies where oat β -glucan, used as a fermentation base with animal stools, similarly boosts SCFA levels. Research by Suzuki and others further confirms that high β -glucan barley consumption leads to increased SCFA concentrations in the cecum of obese mice, highlighting the prebiotic efficacy of this water-soluble dietary fiber, β -glucan. The acetate produced through fermentation in the large intestine is not only an energy source for the liver and peripheral tissues but also a molecular signal for gluconeogenesis and lipogenesis pathways; the propionate produced inhibits fatty acid synthase, thereby helping to reduce blood lipid levels; the butyrate produced provides energy for the intestinal epithelium, conducive to maintaining the integrity of the intestinal mucosa. The intestinal mucosa contains endocrine L-cells that can secrete a variety of peptides, among which GLP-1 and PYY play important roles in regulating food intake and promoting pancreatic function [8]. Research has also confirmed that SCFAs can regulate the expression of the precursor of glucagon-like peptide, which is related to regulating food intake, fat accumulation, and pancreatic function. The fermentation product butyrate can also inhibit inflammatory responses, playing a significant role in apoptosis, cell proliferation, and reducing the risk of colon cancer [10].

3.3. Inhibition of Fat Formation by β -glucan

Managing fat synthesis is a pivotal strategy in combating obesity. It's been documented that subjects consuming β -glucan from barley over six weeks experienced reductions in body weight, waist dimensions, and visceral fat. β -Glucan facilitates this by boosting enzymes responsible for fat metabolism while inhibiting fat-storing enzymes, thereby limiting fat accumulation [11]. Evidence supports that β -glucan's consumption significantly affects fat storage and lipid metabolism regulation, presenting a preventive measure against fat deposition [12]. In research with obese mice administered soluble and insoluble β -glucan, notable improvements were seen in body weight, lipid levels, and other health indicators after six weeks, highlighting β -glucan's potential in improving obesity-related metrics [13,14]. Additionally, a study involving Japanese participants with visceral fat-type obesity demonstrated that a diet rich in β -glucan from barley over 12 weeks effectively reduced body weight, visceral fat, BMI, and waist size, offering a viable option for managing visceral fat-type obesity [15].

4. Conclusion

In summary, numerous studies have shown that regulating the gut microbiota through the consumption of β -glucan can effectively address obesity from multiple perspectives. Firstly, β -glucan can alter the quantity of different microbial populations within the intestine, promoting the growth and activity of beneficial bacteria, thereby modulating the composition of the gut microbiota. Secondly, the fermentation of β -glucan by the gut microbiota produces SCFA products. SCFAs can activate intestinal hormones, effectively influencing the composition of gut microbiota and carbohydrate metabolism, thus reducing the incidence of obesity. Lastly, β -glucan can effectively inhibit the formation of fat. It can reduce the accumulation of white adipose tissue and enhance the activity of certain beneficial enzymes while decreasing the activity of some lipases and proteases, thereby reducing fat accumulation. Overall, researching the mechanism by which β -glucan affects chronic diseases through its action on the gut microbiota holds significant implications for the prevention and treatment of chronic diseases.

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VisRNA: Interactive web server for scRNA-seq data analysis to discover therapeutic targets for non-small cell lung cancer

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Abstract. Motivation: Non-small cell lung cancer (NSCLC) is a leading cause of lung cancer diagnoses and mortality worldwide. Single-cell RNA sequencing (scRNA-seq) has transformed our molecular understanding of cancer by revealing gene dysregulation in individual cells. This granularity allows the discovery of new therapeutic targets, promising NSCLC treatment strategies. Method: The study introduces VisRNA, a Python-based web application for analyzing scRNA-seq data statistically and functionally. VisRNA uses advanced machine learning algorithms to reduce multiple dimensionalities in scRNA-seq data and automatically annotate cell types. This method allows gene expression analysis across NSCLC tumor cell populations. Results: VisRNA analysis of NSCLC scRNA-seq datasets identified 14 distinct cell types with distinct gene expression patterns. VisRNA found several significantly differentially expressed genes in these cell populations that could be therapeutic targets. The application ranked drug candidates by molecular docking simulation performance, indicating potential efficacy against targets. Conclusion: VisRNA is a powerful tool for identifying new therapeutic targets and drug candidates for NSCLC, highlighting a significant advancement in scRNA-seq data analysis. VisRNA helps understand the disease's molecular basis by examining gene expression in individual NSCLC tumor cells, which may lead to better treatments.

Keywords: scRNA-seq, non-small cell lung cancer, visualization platform

1. Introduction

Lung cancer is the second most prevalent and the most lethal type of cancer [1-3]. Specifically, non-small lung cancer (NSCLC) accounts for more than 87% of lung cancer cases. NSCLC begins at the cellular level, where abnormal cells reproduce rapidly. Genetic factors play a crucial role in understanding the mechanism of NSCLC and the prognosis of NSCLC [4-6]. Most NSCLCs are driven by chromosomal instability (CIN), providing genetic diversity to promote cancer progression. These unique attributes collectively contribute to the high complexity and heterogeneity of the cancer genetic landscape [7].

Single-cell RNA sequencing (scRNA-seq) emerges as a high-resolution method for identifying gene expression profiles across different cell types. scRNA-seq examine the gene expression levels in an individual cell by measuring the mRNA expressions. The transcriptomic profile of each cell type differs from the others and exhibits heterogeneity in the tumor microenvironment. This approach is suitable for investigating subcellular-level biology or immune cell heterogeneity [8-9], helping investigators

determine marker genes more efficiently. Consequently, effective visualization and downstream analysis of scRNA-seq data are pivotal for uncovering cancer biomarkers and potential therapeutic targets.

In response to the rapid evolution of computational tools integrating scRNA-seq data of different cancer types and visualization, numerous software applications have emerged for downstream analysis of scRNA-seq. Recently, Zeng et al. developed the CancerSCEM [10-11] database that integrates datasets from scRNA sequencing over the past decade. This dataset encompasses a diverse array of cancers and their sequencing data, offering a user-friendly interface for researchers. Such tools are instrumental in unraveling critical genes indicative of tumor cell heterogeneity. However, only a few studies have focused on finding potential therapeutic targets that may be used for drug repurposing after identifying the marker gene for each cell type. Beyondcell addresses a significant cancer treatment challenge of tumor cell heterogeneity. Variation within tumors often leads to cell responses to treatment options that lead to drug resistance and therapeutic failures. Beyondcell identifies cell subpopulations based on their drug responses to overcome this challenge [12].

I designed an end-to-end web application to perform downstream analysis of scRNA-seq data for the semiautomatic discovery of therapeutic targets of NSCLC in this project. I applied the web app for the drug repurposing of NSCLC. I first performed dimensionality reduction and visualization on NSCLC scRNA sequencing data, followed by cell type annotation using a machine learning approach, differentially expressed gene (DEG) analysis, and functional enrichment analysis. The 10 high-ranking DEGs were identified for each cell type and mapped to drug targets to examine the potential binding affinity with drug candidates of NSCLC. The whole pipeline was hosted on a public web server (VisRNA) with a user-friendly interface, which will promote high-throughput biomarker discovery based on scRNA-seq data and assist drug discovery for NSCLC.

2. Methods

2.1. Dataset

The dataset used in the project was acquired from a publicly accessible database called CancerSCEM [10-11], which contains processed scRNA-seq data in the format of matrices. Specifically, I obtained data from GSE123904 (GEO accession number) with 14 tumor and adjacent standard samples, which provided the gene expression matrices, cell components, differential expression genes, and some critical molecules with the cell interactions. The normalized gene expression matrix of the NSCLC data was extracted for the study.

2.2. Downstream analysis of scRNA-seq data

2.2.1. Visualizing data in low-dimensional space. Three dimensionality reduction methods were implemented on VisRNA: principal component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), and Uniform Manifold Approximation and Projection (UMAP). PCA projects high-dimensional scRNA-seq data into a linearly orthogonal low-dimensional vector space [13-14]. t-SNE converts cell similarities into probability and includes information from cell clusters into visualization by redefining the likelihood. It computes spatial cell maps in low dimensions by minimizing Kullback-Leibler divergence [15]. UMAP creates a high-dimensional graph representation of the data before constructing a low-dimensional graph that is as structurally comparable as feasible. UMAP is well known for its computational efficiency and astounding ability to preserve the global structure of the data itself [16-17].

2.2.2. Clustering cells into putative subpopulations. After dimension reduction, cells with similar gene expressions would be close to each other on the plot and vice versa. Clustering analysis was performed to identify subpopulations of cells.

For PCA and t-SNE, K-means clustering was performed. For UMAP, Leiden clustering [18] was adopted to perform clustering analysis for subsequent cell type annotation. Several critical parameters that may determine the number of clusters are customizable.

2.2.3. Cell type annotation. CellTypist (<https://www.celltypist.org/>) was used to perform cell type annotation, a machine learning-based approach for rapid and accurate cell type recognition created to resolve immune cell heterogeneity across tissues [19]. CellTypist contains a few pretrained models for different tissue types, such as heart, lung, kidney, and organism types, including humans and mice. If some clusters could not be annotated, CellMarker (<http://xteam.xbio.top/CellMarker/>) was used to find the possible appropriate marker gene. As a result, based on DEGs and the CellMarker database, several vital genes overlap in both datasets to determine the cell type for ambiguous clusters [20].

2.2.4. Differentially expressed gene analysis between clusters and cell types. A Welch t-test was performed on the log-expression value for each gene and each pair of clusters to find the marker gene between different cell clusters. The goal is to determine differentially expressed genes (DEGs) by comparing them to the other cells in the cluster. The top DEGs are also good candidates for markers, since they distinguish themselves from clusters. The results of the DEGs are summarized in a table that directly compares the DEGs of each cluster. A heat map was used to show DEGs to visualize gene expression. High-ranking DEGs possess robust and constant up or down-regulation in one of the clusters compared to others.

2.3. Availability and Implementation

The complete source code is available on GitHub (<https://github.com/ryanmxc/VisRNA>). It can be downloaded as a package for offline usage. Additional information on the methods used, including data acquisition, downstream analysis of scRNA-seq data, cell type annotation, differentially expressed gene analysis, gene enrichment analysis, and interactive visualization web server development, is available on the Github repository.

3. Results and Discussion

3.1. Framework of VisRNA for scRNA-seq data visualization

The visualization of scRNA data concerning NSCLC encompasses several essential steps. VisRNA can be hosted locally. All the essential modules for processing and visualization are seamlessly integrated into the VisRNA web server, powered by Python and Streamlit (**Figure 1**). The initial steps involve the extraction of scRNA-seq-processed data from the CancerSCEM database. Subsequently, data dimensionality is reduced through PCA, t-SNE, and UMAP techniques. Then, K-means and Leiden clustering were used to distinguish cell clustering. Furthermore, a hierarchical progressive machine learning model, Cell Typist, with its comprehensive atlas of gene sequences, was utilized to achieve accurate cell-type predictions. This model can also discern marker genes through differential gene expression (DEG) analysis. These marker genes serve as critical indicators for early disease suppression. This platform also generates a heat diagram showing differentially expressed genes in each cell type. **Figure 2** shows screenshots of the essential steps in the VisRNA web application, including data upload, dimensionality reduction, cell type annotation, and DEG analysis. Users can effortlessly upload preprocessed scRNA-seq data in CSV format, entrusting the platform to execute automatic processing. Furthermore, all clustering diagrams, cell type annotations, and DEG analysis results are downloadable for further analysis, including drug–target interaction prediction.

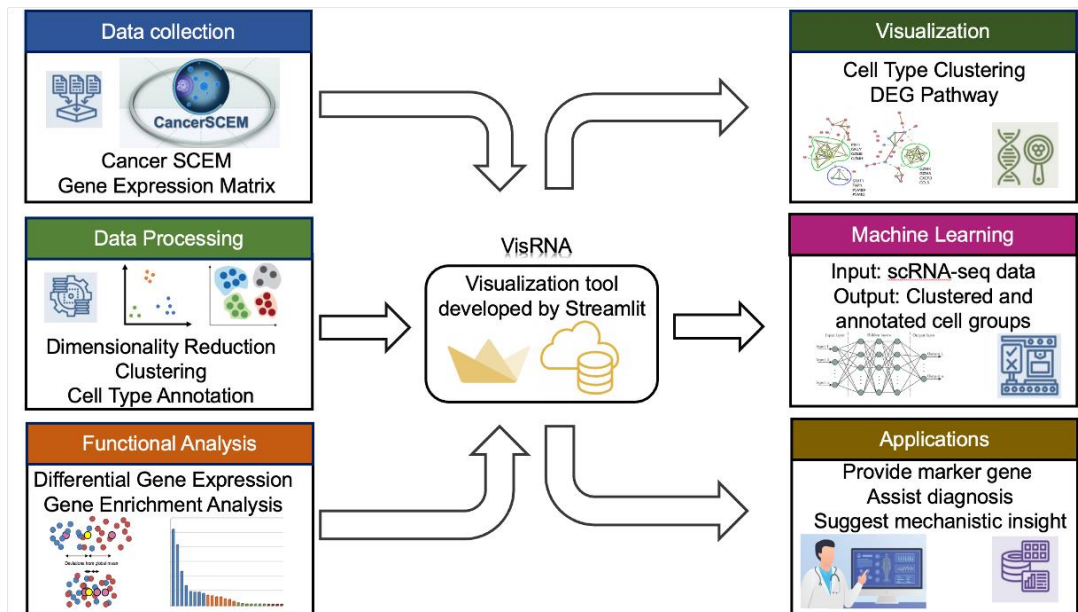


Figure 1. Framework of VisRNA for scRNA-seq data visualization of NSCLC.



Figure 2. Screenshot of VisRNA for scRNA-seq data visualization. Panel A shows the page of load processed scRNA-seq data. Panel B shows the dimensionality reduction page, and the figure can be downloaded using the download button. Panel C shows the cell type annotation done by CellTypist. Panel D shows the differential gene analysis on the webpage.

3.2. Dimensionality reduction and clustering of scRNA-seq data for NSCLC

I employed three dimensionality reduction techniques and different clustering methods in the dimensionality reduction module of VisRNA, as described in the Methods section. Users can fine-tune parameters to determine cluster counts for subsequent cell-type annotations. This study compared PCA, t-SNE, and UMAP techniques for NSCLC scRNA-seq data visualization. The number of clusters ranged from 12 to 17. **Figure 3** demonstrated that t-SNE and UMAP plots formed more concentrated clusters than PCA. Leiden clustering was deployed with UMAP generating the most discrete clusters, a desirable outcome for cell-type annotation.

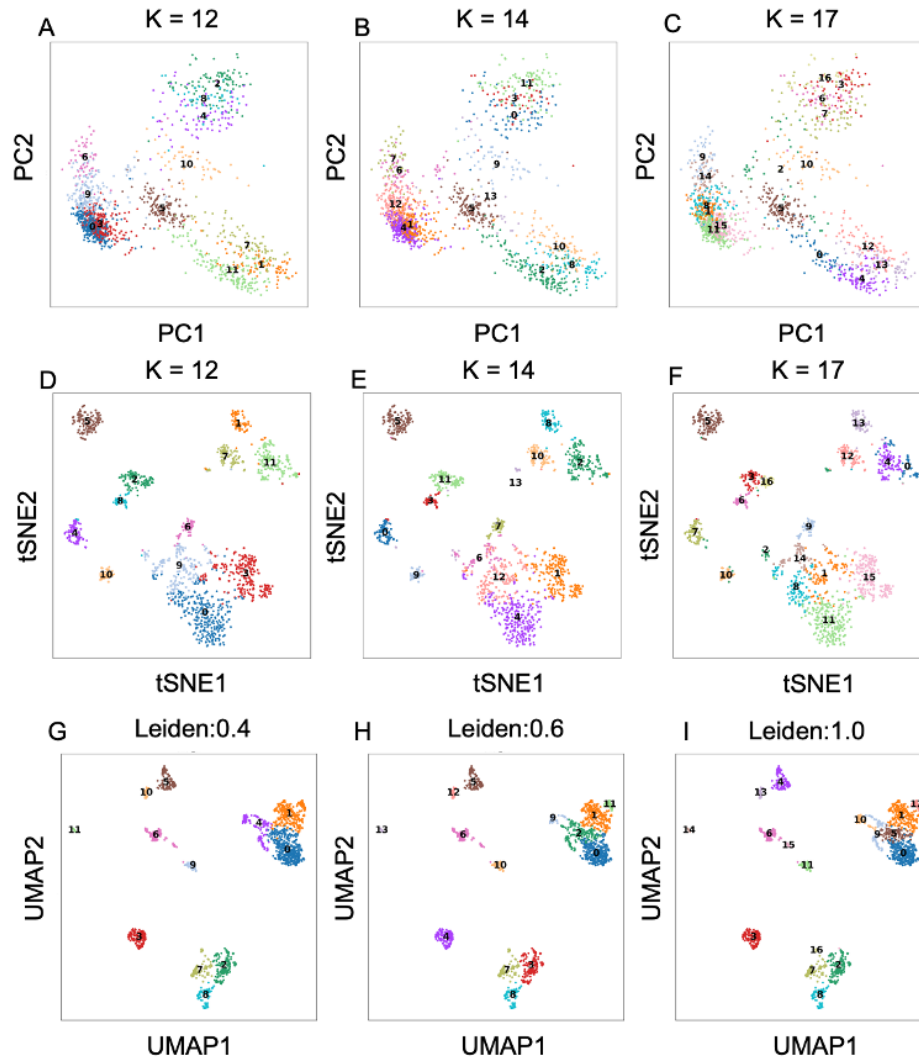


Figure 3. Dimensionality reduction and clustering by PCA, t-SNE and UMAP. Panel A-C shows the dimensionality reduction by PCA and the clusters are divided by K-means into 12, 14, or 17. Panel D-F shows the dimensionality reduction by t-SNE and the clusters are determined by K-means into 12, 14, or 17. Panel G-I shows the dimensionality reduction by UMAP and the clusters are divided by Leiden clustering into 12, 14, or 17.

3.3. Cell type annotation and differentially expressed gene analysis

After performing cell type annotation using CellTypist (<https://www.celltypist.org/>), I utilized known marker genes to visualize gene expression across all cells to validate the reliability of cell type annotation, as shown in **Figure 4**. The marker genes for mast cells and fibroblasts were distinctly located within

their respective clusters, allowing for the confident annotation of these cell types. These cells serve as prime examples of explicit annotation, which helps eliminate ambiguity during the cell-type annotation process. However, certain cell types, such as CD8 Tem and CD8 naive T cells, exhibit similar expression profiles, thus increasing annotation uncertainty. Nevertheless, the putative annotations for these cell types allow for further differentially expressed gene analysis. Each cell type has a few differentially expressed genes. Eventually, 17 clusters were annotated with 14 cell types, with some of the clusters identified as the same.

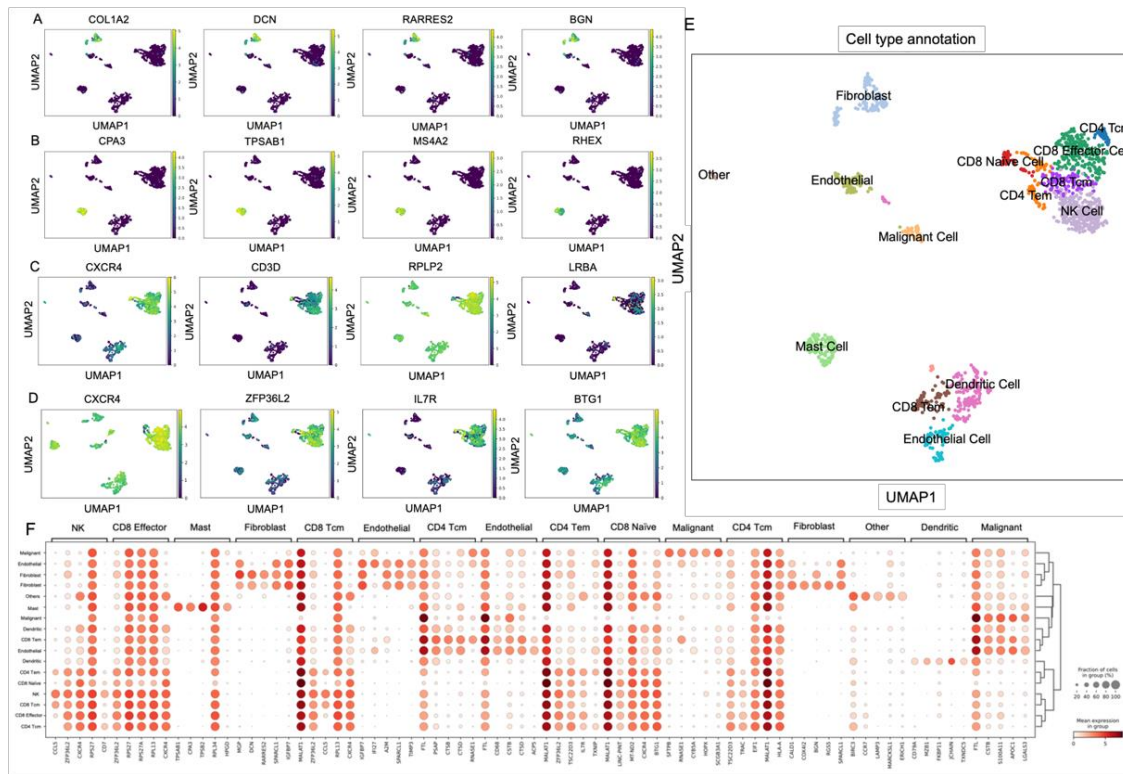


Figure 4. Cell type annotation and differentially expressed gene analysis of NSCLC data. Panel A-D show the feature plot of the most differentially expressed gene in mast cells, fibroblasts, CD8 Tem and CD8 naive T cells in order. Panel E shows the cell type annotation result performed by CellTypist. Panel F shows the differentially expressed gene analysis with annotated cell type.

4. Conclusion

VisRNA harmoniously integrates the procedures for downloading scRNA-seq data, including dimensionality reduction, clustering, cell type annotation, and differential gene analysis, producing a user-friendly interface in which all the data were transformed into visualizations for biological interpretations to find therapeutic targets. The machine learning-based method, cell type annotation, is seamlessly embedded in VisRNA, allowing for rapid identification of prospective therapeutic targets. Differential gene analysis enables rapid identification of potential therapeutic targets. VisRNA is an interactive scRNA-seq data visualization platform for efficient data analysis and therapeutic target discovery.

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Predication on organs-on-chips applications the systematic treatment of breast cancer

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Abstract. With the continuous development of cancer therapy, traditional in vitro models have been unable to reproduce the structure of organs and tissues of various parts of the human body, fluid flow and mechanical stimulation characteristics, and the human tumor microenvironment, which is crucial to the study of the behavior and progress of cancer, can not be presented through the animal model in vivo, which shows that the traditional physiological model of cancer can no longer meet the research of human anti-cancer therapy. Benefits from the continuous infusion of cell cultures through the microchannel, the device of organ-on-a-chip improves cell viability and activity and facilitates the simulation of the tumor cell microenvironment, which is expected to further replace traditional zoological experiments. This study reviews a method to simulate the microenvironment of the vitro breast cancer metastasis combined with microfluidic technology, and the application of this device in relevant researches of breast cancer cell metastasis and screening of breast cancer therapeutics.

Keywords: Organ-on-a-chip, Breast cancer, Cancer metastasis, Drug testing

1. Introduction

As one of the most familiar cancer types, breast cancer mainly occurs in women, which causes numerous women's deaths in the world per year. IARC (International Agency for Research on Cancer) shows from the latest global cancer incidence data that breast cancer surpassed lung cancer for the first time to become the top cancer in the world, and the amount of new cases of breast cancer worldwide reached 2.26 million in 2020, which shows breast cancer becomes a potential threat for women[1]. The uncontrolled proliferation of mammary epithelial cells under the action of various carcinogenic factors is the cause of breast cancer. Breast cancer occurs mainly in women and is often referred to as the "pink killer." [2].

In 2016, on the World Economic Forum's list, human organ-on-a-chip technology was one of the "top ten emerging technologies." Organ-on-a-chip is a revolutionary method that could one day replace animal testing [3]. The organ chip provides a highly bionic breast organ unit. Compared with traditional animal experiments, the microfluidic system has high data acquisition efficiency and can better simulate the actual situation of human organs. It has the advantages of strong replication, avoiding human interference, saving economic costs, and so on. Besides, organ-on-a-chip can detect the dynamic, spread, and metabolic activities of tumor cells in human functional tissues and organ environments in vitro, which plays an important role in drug toxicity analysis, tumor modeling, and

drug metabolism. [4]. This paper reviews the in vitro culture of breast cancer cells based on organ chips, commonly used in vitro models of breast cancer cell metastasis, and several basic medicine tests.

2. In Vitro Culture of Breast Cancer Cells

Two-dimensional cell culture plays a crucial part in cancer research, but it has a good deal of limitations because it cannot simulate the microenvironment of the original tumor and cannot accurately represent tissue cells in vitro. For example, because the cell can only grow and expand in two dimensions, the cell is slender and flat and cannot be preserved in its natural form [5]. In addition, there are some differences in cell differentiation, drug metabolism and gene expression. Three-dimensional cell culture overcomes some of the limitations of traditional two-dimensional cell culture, reducing the differences in drug screening between in vitro and in vivo models and reducing the possibility of using animal models for experiments. But they were unable to reproduce the chemical and physical characteristics of the tumor microenvironment, lacking the fluid flow, tissue deformation, and shear stress that play important roles in cancer cell invasion. Animal models are ethically controversial, expensive, and do not predict the actual effects of drugs on humans [6].

System-on-a-chip is a convenient and versatile approach that can be applied to simulating the functions of various organs in the human body, enabling the implantation of human cells, creating patients's specific multicellular settings for conducting personalized medical research, and providing a platform for studying the interaction of actual organs with proposed therapies. In recent years, the in vitro culture of breast cancer cells based on organ chips has been successfully put into experiments and made relevant progress.

The researchers cultured human breast cancer cells with a combination of immortalized human breast fibroblasts and a variety of extracellular matrix factors in a microfluidic device [7]. The results showed that human breast fibroblasts promoted the growth of breast cell clusters compared to monocultures, especially in the presence of an extracellular environment rich in fibronectin. Factors that influence tumor growth also include changes in the levels of cytokines secreted by adjacent normal parenchymal cells in the local tissue microenvironment.

3. In Vitro Model Construction of Breast Cancer Cell Metastasis

Tumor metastasis is a multi-step complex process, which refers to the process in which cancer cells transfer from the original tumor site to distant tissues and organs to form other tumor sites. Almost 90% of cancer patients die due to tumor spread[1]. Breast cancer cells metastasis is strongly linked to the primary microenvironment and other tumor positions, and sufficient interaction between the microenvironment and tumor cells is crucial for whether tumor cells can survival. However, the common two-dimensional tumor model cannot accurately and intuitively reflect the three-dimensional structure of the tumor inside the human body, how the tumor cells interact with each other, and the related mechanisms of life activities such as cell genetics and metabolism in tumor microenvironment. Therefore, the multi-organ interaction and real-time monitoring of dynamic, functional and physiological responses of living organs are realized by using organ chips.

3.1. Metastasis Mechanism of Breast Cancer Cells

Tumor cells initiate the metastasis cascade by interacting with other organ microenvironments at the cell-to-cell and cell-to-extracellular matrix levels. The metastatic cascade, which means to spread tumor cells from the original tumor position to other distant organs, is a dynamic and intricate process. The occurrence of metastasis begins when the cancer cells begin to migrate to the surrounding matrix. Then they enter the vascular system and metastasize to other organs to form secondary tumors. Some cancer cells attach to the vessel walls after entering the vessel circulation and exude into the new microenvironment of the target organ. Cancer cells adjust and eventually form new tumors in the tissue environment at the secondary site. The metastasis of breast cancer is closely related to its subtype.

The types of Breast cancer include luminal A, luminal B, triple-negative, and HER2-positive types, and the classification of breast cancer is relevant to hormone receptors and cells. When using organ-chip to study breast cancer metastasis, liver, lung, brain and bone are considered as potential metastatic sites, among which luminal A and luminal B tend to metastasize to bone, HER2-enriched breast cancer mainly leads to liver metastasis, while TNBC subtype has a higher tendency to lung metastasis [8].

Bersini's team created a three-dimensional microfluidic model to analyze bone metastasis specificity in human breast cancer [9]. Through this system, the extracellular matrix of proteins secreted by bone-differentiated cells can better simulate the complex signals in the cellular microenvironment, generating the microenvironment of muscle and acellular collagen. Due to the components secreted in the bone microenvironment that promote the exosmosis of breast cancer cells, tumor cells can specifically move toward the bone. These results suggest that tumor cells have a tendency to seek an acceptable microenvironment to maintain proliferative growth and the process of tumor metastasis. Through the construction of an organ-on-chips model, the human tumor microenvironment is better simulated, which is conducive to the study of the metastasis mechanism of the tumor cell microenvironment.

3.2. Common In Vitro Construction Models based on Organ Chips

In addition to studying how breast cancer cells metastasize, organ chips can also simulate the process of breast cancer cells invade to other organs and cells [10]. In one study, the researchers simulated the invasion of breast cancer under the action of macrophages. A three-dimensional breast cancer in vitro invasion model was created by using a microfluidic system. Prepare two channels, fill them with 3D matrix gel before placing the tubes in parallel. Macrophages stained with red fluorescent protein and tumor cells stained with green fluorescent protein were implanted separately. Put HUVECs suspensions in nutrient supply channels to form a single permeable vascular endothelial barrier to simulate blood vessels of mammals. The total migration distance can be determined by connecting the channels between macrophages and tumor cells through the gaps between microchannels and calculating the number of tumors migrating to adjacent channels. The system mimics the arrangement of microorgans in the body, recreating a more biomimetic external environment. There is also a 3D model consists of a cell-filled hydrogel, creating an endoderm model of breast cancer cells by replicates tumor cells stroma and blood vessel networks. The model shows that breast cancer cells exhibit different aggressiveness depending on their ability to metastasize and whether a vascular layer presence or not.

The organs-on-chips model can simulate the coordination and internal connection of multiple organs as well. Nowadays, researchers have already achieved long-term co-culture of skin, intestinal, liver, kidney and other organoids on a chip, and establish a multi-organ chip with the combination of various organs [11]. Besides, the combination of chip and multi-mode sensing technology is the future development trend to realize the information acquisition and processing of multi-organ chip. A multi-organ chip, with integrated electrochemical and immune sensing modules, can synchronously monitor cell microenvironment parameters such as carbon dioxide concentration, power of hydrogen and temperature, as well as some biomarkers that reflect tissue function.

4. Drug Testing and Analysis of Breast Cancer Cell Prevention and Treatment based on Cell Chip

In traditional drug testing, due to the complexity and heterogeneity of tumors, patients often have different therapeutic effects under the same drug treatment, so it is difficult to determine the best treatment plan for each patient. By reconstructing the genetic and genetic signatures of the primary tumor, organ chips have great potential for medicine response testing in several given cancer subtypes with unique genome mutations. Moreover, through the construction of multi-organ chips, the metabolism and influence of drugs can be better observed, and the possible side effects of drugs can be screened for patients.

In one study, triple-negative breast cancer cells were exposed to a series of anticancer drugs (olaparib, fir, cisplatin, etc.) to characterize the inoculation density, ECM composition and biochemical conditions, based on an organ-on-a-chip platform for the testing of breast cancer treatment methods [12]. The results showed that the response of organ-on-a-chip model cell lines to cisplatin was more similar to physiological response. Therefore, this technology is a promising individualized medical tool, which is conducive to patients to choose the appropriate treatment method. In the process of drug development, organ chips can also be used to achieve the screening of different concentrations of drugs, greatly saving screening time. Eduati's team created a microfluidic platform that can quickly examine a variety of medicine combinations in cell analysis[13]. On this platform, Braille valve chip was mounted the on the Braille display, using an internal bracket to press the dimethylsiloxane chip onto the Braille pin. A program designed according to a predefined sequence corresponding to the system's drug binding and barcode controls the movement of each Braille pin. By adding organoids from pancreatic tumors to the system, 62 conditions generated in turn were used to test the overall efficacy of each drug under all combinations. Using this device, the effectiveness of single sensitive medicine or multi-medicine combination chemotherapy regimen can be effectively detected, and the efficiency of drug detection can be greatly improved. Compared with the traditional model, this method can detect different drugs quickly and accurately at the same time, and multiple gradient tests can also be conducted to determine the concentration of anticancer drugs, which can improve the efficiency of future cancer drug research and development.

This paper argues that drug testing on an organ-on-a-chip should be accompanied by an evaluation of drug absorption, distribution, metabolism, excretion, and toxicity of chemotherapy, immunotherapy drugs, or radiation. If necessary, multiple organ functions can be reconstructed on the chip platform, so that the multiple organs and tissues that need to be observed are connected together, and the metabolic network similar to that of individual or combination medicine in human bodies can be built. The construction of in vitro models of multiple organ connections can provide a better understanding of drug properties, better potential side effects and prediction of efficacy in new drugs and treatment events, and provide a precious integration of regenerative medicine and translational science.

5. Conclusion

Through the process of breast cancer cells cultured in vitro, a highly bionic breast organ chip is constructed, which can better simulate the human tumor microenvironment and realize further research on the metastasis mechanism and invasion process of breast cancer cells. At the same time, organ chip also greatly improves the screening of different drugs and drug concentrations for breast cancer treatment, and simulates the metabolic process of drugs through multi-organ chip to detect the possible side effects of drugs on patients to the greatest extent. The shortcoming of this paper is that it only reviews the treatment method and diagnosis of breast cancer, which has not been integrated with the research on organ chips such as lung cancer and liver cancer, and the number of review articles is limited. Nowadays, there are still many deficiencies in the field of multi-organ chip, there is no clear distinction between the concept of organ chip and organoid chip, and the standards for drug testing on organ chip are not perfect. With the gradual maturity and specification of organ-on-chip technology standards, it will have further comprehensive applications in precision medicine and biodefense strategies.

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Association between circadian gene expression amplitude and phase abnormalities and ocular diseases

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Abstract. The biological clock is an internal timing regulation system that exists in almost all organisms, including humans. It plays an important regulatory role in the physiology, behavior, and cognitive functions of organisms. It is one of the important mechanisms for organisms to adapt to the environment, maintain a stable internal environment, and achieve coordination of biological functions. The master controller of the biological clock system is located in the suprachiasmatic nucleus of the brain. Normal operation of the biological clock requires the normal expression of clock genes such as *Bmal1* and *Cry*. Clock and rhythm genes also exist in the retina. Damage to the retinal clock can affect the occurrence and development of retinal diseases and also have an impact on retinal aging. This article reviews the relationship between myopia, diabetic retinopathy, glaucoma, age-related macular degeneration, and abnormalities in clock genes, providing new ideas for the management and treatment of these diseases in clinical practice.

Keywords: Circadian gene, Myopia, Diabetic retinopathy, Glaucoma, Age-related macular degeneration

1. Mechanism of Circadian Rhythms

For their contributions to the discovery of molecular processes governing circadian rhythms, Jeffrey C. Hall, Michael Rosbash, and Michael W. Young were jointly granted the 2017 Nobel Prize in Physiology or Medicine. Circadian rhythms are broadly distributed at the cellular level in a wide range of animals, including bacteria, algae, and vertebrates. These rhythms help these organisms adjust to recurring environmental changes, such as the 24-hour day-night cycle [1]. This environmental variation serves as a major factor influencing circadian rhythms. The central clock regulating biological circadian rhythms is located in the suprachiasmatic nucleus (SCN) which is in the hypothalamus. In mammals, the eyes constitute the only light signal receivers for the SCN. Three different types of photoreceptor cells in the retina—rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs)—are involved in the functioning of this central clock [2].

In mammals, various systems such as endocrine and cardiovascular systems exhibit rhythmicity. Melatonin, for instance, reaches its peak at night and then falls in the presence of light to encourage awakening. It rises before nightfall as a result of lowering light intensity [3]. Blood pressure level rises

during the morning and decreases at night, which is known as the dipper pattern [4]. The retina also possesses rhythmicity, and ocular physiology is influenced by circadian rhythms. ipRGCs play a crucial role in retinal rhythms. Atypical ganglion cells called ipRGCs play a role in circadian rhythm regulation, pupillary light reflex modulation, and visual development. ipRGCs express melanopsin, allowing them to respond to light signals beyond input from cones and rods [5]. ipRGCs integrate light signals processed and sent by rods and cones and send axons to the SCN and several other locations in the brain [6]. When ipRGCs process light signals, both wavelength and irradiance act as regulatory signals to make sure the circadian rhythm system is in sync with the outside light/dark cycles [7]. Circadian rhythm regulation allows vision to adapt to the alternating day-night cycle (i.e., variations in light intensity) and also regulates the retina's ability to send signals to the circadian rhythm system. Extensive research has been conducted on retinal circadian rhythms within 24 hours, and it is now known that many molecular and cellular processes are controlled by the biological clock. Increasingly more "clock genes" and unique transcriptional-translation feedback pathways have been described in mammals. Although there are some differences, they still adhere to the same basic principles: light signals regulate the expression of numerous rhythm genes.

In summary, the regulation of rhythm gene expression involves two branches: (1) direct transcriptional activation of Clock-Controlled Genes (CCGs) genes due to the cumulative response of clock factors and tissue-specific regulatory factors; (2) indirect effects originating from transcription factors acting as clock targets [8]. Specifically, circadian rhythms rely on transcription factors encoded by genes such as *Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1*, and *Cry2*. These clock genes interact through a series of transcription-translation feedback pathways. The CLOCK:BMAL1 heterodimer interacts with clock genes *Period 1-2* (*Per 1-2*) [9] and *Cryptochrome 1-2* (*Cry 1-2*) [10], as well as the E-box enhancers of CCGs, promoting their expression [11]. After the interaction between PER proteins and CRY proteins, they will be transported to the nucleus, where they can interact with the CLOCK:BMAL1 heterodimer, inhibiting its transcription, thereby suppressing their transcription [12]. The second feedback loop involves in REV-ERB proteins and Retinoic acid-related orphan nuclear receptors (RORs). CLOCK:BMAL1 also activates the nuclear receptors *REV-erba* and *REV-erbβ*, which competitively bind to RORs on the ROR-binding elements (ROREs). The expression of *REV-erba/β* inhibits the expression of *Bmal1* [13], while *ROR* can promote *Bmal1* expression.

Increased exposure to artificial light sources at night disrupts the organism's biological clock. Existing research indicates that circadian rhythm disruption may increase the risk of cancer, metabolic diseases (such as obesity, and diabetes), neurological disorders (like Alzheimer's disease), psychiatric disorders (such as bipolar disorder, depression, and anxiety), cardiovascular diseases (such as myocardial infarction, arrhythmias), skeletal and muscular diseases, and autoimmune diseases [14]. Variations in light-darkness are an important feature of circadian rhythms. The eyes are the most important photoreceptive organs in the body and are also regulated by circadian rhythms. Changes in light signals or abnormalities in rhythm genes can affect the structure and physiological functions of the eyes, making them an important area of concern.

In the molecular pathways of circadian rhythms, *Bmal1* is a necessary component of the mammalian circadian pacemaker. Deletion of *Bmal1* results in the immediate disappearance of circadian rhythms, impaired activity during light/dark cycles, and decreased activity levels [15]. Meanwhile, in *Bmal1* knockout mice, changes in corneal structure have been observed, including corneal neovascularization, keratinization, and inflammatory responses [16], leading to a significant increase in the incidence of cataracts and corneal inflammation during aging. Additionally, the vitality of photoreceptor cells is significantly reduced [17]. These changes can lead to the occurrence of certain eye diseases. Circadian rhythm regulation in the retina is present in all vertebrates, but significant differences exist in the circadian rhythms of the retina among different taxa and usually within the same species. The understanding of human circadian rhythm control from the viewpoints of genetics, chemistry, and biochemistry will be clarified in this review, along with the connection between aberrant circadian rhythm genes and eye disorders.

2. Circadian Genes and Eye Diseases

2.1. Circadian Genes and Myopia

Myopia is a common global ocular disease, mainly occurring during childhood and adolescence, characterized by blurry distant vision. Research on children's eye diseases in Anyang found that the annual incidence and progression rates of myopia and high myopia are high among school-age children in China, especially after the third grade, where the incidence of myopia increased from 7.8% in the first and second grades to 25.3% in the fifth and sixth grades, and the incidence of high myopia increased from 0.1% to 1.0% [18]. It is estimated that by 2050, half of the world's population will be myopic [19]. Myopia can be divided into axial myopia and refractive myopia, the former is associated with an excessive axial elongation of the eye, while the latter is associated with an excessive curvature of the cornea or lens [20]. The primary causes of myopia in the majority of cases are contemporary lifestyle choices such as extended near-distance work and little time spent outside. However, genetic factors are also significant. [20].

Meta-analysis has demonstrated that light-dependent retinal signals are important driving factors for refractive errors [21]. Additionally, large-scale GWAS studies have found circadian rhythm genes, such as *CRX*, *PAX4*, and *OTX2* [22], to be associated with the occurrence of refractive errors and myopia in European populations [23]. The irregularities of modern lifestyles promote the development of the disease. Both the axial length of the eye and the thickness of the choroid undergo circadian rhythm changes as myopia develops. While the choroidal thickness changes in the opposite direction, the axial length achieves its highest value in the afternoon and lowest value in the morning. Remarkably, myopic patients show fewer changes in choroidal thickness but larger variations in axial length [24].

Myopic defocus disrupts the rhythmicity of axial length and choroidal thickness in the human eye. The peak values of axial length and choroidal thickness are found to be delayed by roughly 6 and 8.5 hours, respectively, following myopic defocus. These findings suggest that circadian-related regulatory mechanisms play a role in the alterations of biological parameters in the human eye [25]. Mice can develop myopia if the *Bmal1* gene is specifically knocked off in the retina. Compared to control *Bmal1*^{-/-} and *Bmal1*^{+/-} mice, the gene knockout mice show elongation of the axial eye length and vitreous cavity. Additionally, these mice exhibit slightly decreased contrast sensitivity, which may manifest as central visual problems in humans [26]. This data implies that the development of myopia in the human eye may be influenced by circadian rhythms and genes. Optical defocus disrupts the rhythmic expression of clock genes in the retina/retinal pigment epithelium cells and choroid. In chick form-deprivation experiments, the rhythmicity of 4 genes (*Opn4m*, *Clock*, *Npas2*, and *Mntr1a*) disappeared, and there were time-phase shifts in the occurrence of some clock genes [27]. Outdoor exposure can inhibit form-deprivation myopia in chicks [28].

Based on the above research, the mechanism that can suppress the development of myopia by increasing outdoor activities for adolescents involves the regulation of circadian gene expression. Additionally, reducing the intensity of artificial light exposure at night while increasing daytime activities can enhance the signal of circadian rhythms, thereby increasing the inhibitory effect of outdoor exposure on myopia [29].

2.2. Circadian Genes and Diabetic Retinopathy

Diabetic retinopathy is a particular type of microvascular complication, with retinal arteriolar dilation possibly being an early physiological marker of microvascular dysfunction [30]. It manifests as damaged retinal vessels, leading to the formation of ischemic areas and capillary leakage, thereby resulting in macular edema [31]. Diabetic retinopathy is a common complication of diabetes which is a major cause of preventable blindness in the adult working population. Its global morbidity is estimated at 22.27%, with the highest prevalence in Africa (39.50%) and North America and the Caribbean (33.30%) [32]. In China, about 40% of diabetic patients suffer from diabetic retinopathy [33]. Globally, there are projected to be 103.12 million patients with diabetic retinopathy by 2045 [32]. According to studies, type 2 diabetic female patients have a higher incidence of diabetic retinopathy than male patients do, while

male patients also have more severe retinopathy and visual impairment or blindness [34]. Higher blood glucose levels, hypertension, the duration of diabetes, and elevated glycated hemoglobin levels are all associated with the prevalence of diabetic retinopathy.

Abnormal expression of circadian genes has drawn a lot of interest in animal investigations of diabetic retinopathy, and disruption of circadian rhythms may be causally related to the lesion's progression. The expression of multiple clock genes is altered in the retinas of diabetic mice. Although *Bmal1* maintains its rhythmicity, its amplitude is dramatically attenuated. Studies have demonstrated that wild-type male C57BL/6J mice were administered streptozotocin at 3 weeks of age and euthanized after 12 weeks to obtain retinal tissue. The expression amplitude of *Per1* and *Cry1* genes exhibited a clear circadian rhythm increase, while *Per3* lost its rhythmicity [35]. However, in another experiment, male Long Evans rats received streptozotocin injections at 8 weeks of age and were analyzed after 6 weeks, revealing reduced *Per1* expression and elevated *Bmal1* expression in the retina. These findings may be attributed to species or experimental design variations [36].

The disorder of retinal rhythms can also lead to diabetic retinopathy. For instance, *Clock* and *Bmal1* conditional knock-out mice ultimately developed type 1 diabetes. Mice with *Per2* mutations also display symptoms associated with diabetic retinopathy, including acellular capillaries, increased retinal permeability, and progressive retinal vascular dysfunction, along with inadequate endothelial nitric oxide synthase synthesis, upregulation of pro-fibrotic genes, disruption of tight junctions, and decreased adherent junction integrity [37-38]. Research has indicated that fatty acid oxidation damage in diabetic retinopathy may be caused by the disruption of circadian genes and the expression of genes involved in lipid metabolism, which in turn may impact the disease's course [36,39].

Moreover, it is hypothesized that a harmful role for the phase mismatch between internal biological rhythms and retinal metabolic cycles may exist in the development of diabetic retinopathy. It is believed that the phase difference between retinal metabolic pathways and the biological clock governed by light-dark rhythms increases oxidative stress and neural damage, thereby leading to the progression of diabetic retinopathy [40]. Additionally, changes in the retina and retinal clock can also affect the main clock of SCN and its function [41]. In the SCN of diabetic mice, the expression of all circadian genes is reduced [42]. Research has revealed that the phase of *Cry1* expression is delayed, the rhythms of *Cry2* and *Rorb* vanish in the SCN of diabetic mice, and the reduced production of light-induced clock genes in the SCN can also cause morphological alterations in ipRGC [35]. This can also impact retinal rhythm in turn at the same time.

In conclusion, changing the way circadian genes are expressed may also be a strategy for treating diabetic retinopathy. The *Bmal1* retinal clock system is both a target and an effector in the process of diabetes. Although the expression of *Bmal1* is altered and affects retinal rhythm, the loss of *Bmal1* can improve the pathophysiological condition of the retina in mice with pre-diabetes, so *Bmal1* may serve as a potential therapeutic target for diabetic retinopathy [43]. Additionally, REV-erb agonists can significantly improve lipid and hyperglycemia conditions, thereby alleviating disease progression [44]. Chuanxiong ketone acts on ROR nuclear receptors, establishing a connection with the circadian gene network through the REV-erb/ROR pathway, stabilizing the stability of the feedback pathway, and ultimately improving blood sugar and glucose tolerance, playing a therapeutic role [45]. KL001 can also control blood glucose by stabilizing CRY protein [46].

The interaction between circadian genes and diabetic retinopathy is complex, and choosing different experimental animals and analyzing them at different modeling stages may lead to varied experimental results. Therefore, the effects of diabetic retinopathy on circadian genes may be diverse in different periods. In addition to existing drugs that enhance the expression of circadian genes, it is indispensable to conduct relative research on applying specific frequencies and intensities of light to patients to enhance the expression of circadian genes for therapeutic purposes. Whether circadian genes have a clear impact on the onset of the disease still needs further exploration, which is crucial for the management of subsequent diseases.

2.3. *Glaucoma and Circadian Rhythms*

Glaucoma is the second leading cause of blindness worldwide, with higher incidence rates in females and Asians [47]. By 2040, the estimated prevalence of glaucoma is expected to reach 1.118 billion people [48]. Glaucoma is characterized by progressive degeneration of a group of retinal ganglion cells in the optic nerve [49]. Research has largely concentrated on the function of intraocular pressure (IOP), while also taking into account other aspects such as aberrant ocular blood flow and the lamina cribrosa's pressure tolerance, even if the pathophysiology of glaucoma is not entirely known [50]. Risk factors for glaucoma include aging, non-white ethnicity, and a family history of glaucoma [51-53].

It has long been known that intraocular pressure follows a rhythm, peaking at night and falling during the day [54-55]. This rhythmicity depends on circadian genes and the integrity of the circadian clock, with known involvement of *Cry* genes in generating the diurnal IOP rhythm [56]. The iris-ciliary body complex has been shown to express circadian genes, which may regulate intraocular pressure [57-58]. Current research indicates that IOP is driven by the rhythm signals from SCN through cortisol or adrenaline [59]. Translaminar pressure, or the difference in pressure between intracranial and IOP on the papilla of the optic nerve, may cause damage to retinal ganglion cells as IOP. This is one of the pathogenic mechanisms of normal-tension glaucoma, according to certain studies [60]. Similarly to intraocular pressure, intracranial pressure also exhibits rhythmicity [61], peaking at night. Additionally, nighttime blood pressure reduction is a risk factor for progressive visual field loss in glaucoma. But unlike intracranial and intraocular pressure, blood pressure is lowest at night [62], which increases the risk of injury from inadequate ocular perfusion [54].

Few studies have been conducted to determine if circadian gene mutations cause glaucoma or if the condition is caused by changes in circadian gene expression. The prevailing theory still attributes glaucoma to pathological alterations in ocular structures as well as the synergistic impact of specific hereditary and environmental variables. Perhaps intervention in daily behaviors to enhance circadian stability could provide some help in controlling the disease.

2.4. *Age-Related Macular Degeneration (AMD) and Circadian Rhythms*

Age-related macular degeneration is the leading cause of incurable blindness in the elderly worldwide. Among the global population, Europeans have the highest prevalence worldwide, and as the world's population ages, it is predicted that 288 million people will suffer from AMD by 2040 [63]. Typical signs of AMD include choroidal neovascularization and drusen. Its pathogenesis is proven to be related to the aging of the retinal pigment epithelial cells, oxidative stress, inflammation, immune responses, and changes in lipid metabolism [63-64].

There is limited research on the role of circadian gene abnormalities in the development of AMD, but studies have shown that as age increases, biological rhythms become increasingly disrupted, and the decrease in rhythm amplitude may be the most severe effect of aging on biological rhythms [65-66]. Meanwhile, the impairment of the retinal clock may also be involved in the development of retinal diseases [67]. During aging, changes in retinal rhythms reduce the survival of photoreceptors. Studies have shown that mice with *Bmal1* knockout have a 20-30% reduction in the nuclei of the outer nuclear layer, while mice with *Clock/Npas2* knockout also show a significant reduction in the photoreceptor layer [17].

One of the key pathogenic mechanisms of AMD is oxidative stress [68], and the circadian clock is also necessary for regulating the body's redox reaction [69]. Anomalies in the WNT/ β pathway are linked to the development of exudative AMD, and anomalies in *Bmal1* function can cause the downregulation of genes in the WNT/ β pathway, which increases the production of lipids [70]. Additionally, *Bmal1* regulates the amounts of reduced FAD and oxidative NADPH [71]. Furthermore, there is a rhythm to the phagocytosis of retinal pigment epithelium cells and the shedding of photoreceptor outer segment discs that is regulated centrally by the SCN and locally by retinal rhythms [71]. Once this rhythm is disrupted, the function and survival of photoreceptors are inevitably affected, and the delayed phagocytosis of retinal pigment epithelial cells leads to the accumulation of substances

within the cells, which is harmful to both the retinal pigment epithelial cells and eventually the whole retina, thus leading to the occurrence and development of AMD [72-73].

Endogenous neurohormone melatonin is generated by the pineal gland and retina. It is a vital rhythmic hormone in retinal rhythms and has an anti-oxidative stress effect on retinal pigment epithelium cells. It has been found that the level of melatonin is significantly reduced in patients with AMD [74]. Studies have shown that daily administration of melatonin can protect the retina and delay macular degeneration [75]. Additionally, blue light stimulation can affect biological rhythms, thereby disrupting the diurnal secretion rhythm of melatonin. Using blue light-blocking lenses to reduce reactive oxygen species and increase antioxidant enzyme levels can help prevent retinal phototoxicity [76].

The relationship between the pathogenesis of AMD and circadian gene abnormalities is worthy of enough attention. The mainstream treatment approach mainly focuses on delaying progression, such as the use of lutein and anti-angiogenic drugs. Currently, there aren't many therapies that target circadian rhythms; however, as was previously noted, lowering blue light stimulation to safeguard the retina is a workable solution, but further study is needed to determine its therapeutic efficacy.

3. Conclusion

The growth and development of organisms rely heavily on biological rhythms, which also guarantee the timely occurrence of physiological responses in the body. The molecular basis for this is the set of biological rhythm genes, including *Clock*, *Bmal1*, *Cry*, *Per*, and others. Among these, *Bmal1* holds particular significance. The four forms of eye disorders that are covered in this paper are myopia, diabetic retinopathy, glaucoma, and age-related macular degeneration. These diseases are related to abnormalities in rhythm genes. Abnormal expression levels and timing of gene expression can trigger changes in diseases, while diseases can interfere with the expression of rhythm genes in turn. The interaction between eye diseases and gene expression is complex. For these eye diseases, several means of treatment, including medication and physical therapy, were already applied and proven to be effective in increasing rhythm amplitudes and stability. In future research, further exploration of abnormalities in the feedback pathways of biological rhythms and their effects on the occurrence and development of diseases should be conducted. Additionally, drugs targeting biological rhythms could serve as a direction for further drug research and development.

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Research on the functional properties and extraction methods of dietary fiber

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Abstract. Dietary fiber is a form of polysaccharide that does not yield energy and cannot be fully broken down and absorbed by the digestive enzymes in humans. Furthermore, the human body is unable to produce it; instead, food produced from plants is the only source of it. There are specific species of gut microbiota that ferment dietary fiber, producing short-chain fatty acids, monosaccharides, and gases as byproducts. Dietary fiber has both physicochemical (solubility, viscosity, absorption, water holding capacity, and oil holding capacity) and functional properties (glucose adsorption, cholesterol adsorption, and nitrite ion adsorption). Dietary fiber can be extracted from plant using a variety of techniques, including chemical, mechanical, and enzymatic methods; however, these techniques have significant shortcomings, such as narrow extraction zones and poor extraction quality. Through the course of the last few centuries, the amount of dietary fiber has significantly decreased, which has led to unhealthy human conditions like obesity, cancer, type 2 diabetes, and constipation. Nowadays, these Dietary fibers, therefore, are given great attention because of their physicochemical and functional properties which are vital for human health. These products from plant resources and fruits can be further used for food manufacturing and processing.

Keywords: Dietary fiber; physicochemical properties; functional properties; type 2 diabetes; intestinal health

1. Introduction

Dietary fibre(DF) is a complex polysaccharide of plant origin that is not hydrolysed endogenously in the small intestine, but is totally or partially fermented by the intestinal microflora in the large intestine. The major source of dietary fibers is extracted from whole grains, vegetables, fruits, and legumes. Dietary fibre promotes the health of the gastrointestinal tract by regulating its composition and metabolism, providing energy to the colonic epithelium, stimulating fibre synthesis, promoting peristalsis and maintaining the integrity of the intestine [1]. Furthermore, dietary fiber can also manage weight by increasing bulk to the diet. In terms of cardiovascular diseases and diabetic patients, dietary fiber can reduce absorption of glucose and regulate blood pressure to decrease the incidence of heart disease.

When dietary fibre is broken down by the intestinal microbiota, short-chain fatty acids (SCFA) such as acetic acid, propionic acid and butyric acid are produced, as well as gases such as methane and carbon dioxide [2]. SCFAs are absorbed by the colon, muscles, liver and other tissues and provide the body

with basic energy in the form of nutrients. Acetic acid facilitates the production of ATP through the citric acid cycle [3].

In recent decades, people have become more aware of nutrition and health. At the same time, it has been recognized that dietary fibre plays an important role in human nutrition and in the fight against chronic diseases. In addition, most people's daily intake of DF cannot meet the requirements of the Dietary Guidelines for Chinese residents, and most Americans consume less than half the recommended daily intake of DF; the daily intake of DF can be as little as 1/3 to 1/2 of the intake recommended in the dietary guidelines [4]. Dietary fiber, therefore, is extracted from plants to be utilized in diet as functional food. This involves chemical, physical and biological processes that can break down glycosidic bonds to a certain extent.

2. Classification and sources of dietary fiber

2.1. Classification of dietary fiber

Dietary fibers are the edible portions of plants and their extracts, as well as synthetic substitutes that are frequently fermented in the large intestine to some extent rather than being absorbed and digested in the small intestine [5]. Based on its solubility, dietary fiber is often divided into two categories: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Dietary fibers are also divided into plant fibers and animal fibers, depending on their origin, while plant fibers are divided into cereal fibers, fruit fibers and vegetable fibers [2].

2.1.1. Soluble dietary fiber: Non-starch polysaccharides, or SDFs, are found in plant cytosol and interstitial fluid and are soluble in water. They can be fermented by the microbiota in the human gut and are typically derived from cereals, fruits, and vegetables [6], such as legumes, vegetables, pectin-resistant dextrin, and psyllium. Simultaneously, the gut microbiota in the human colon has the ability to ferment and breakdown SDF, producing a large number of SCFAs, which are viscous and can form a gel, delaying gastric emptying time and thus reducing cholesterol storage [7].

2.1.2. Insoluble dietary fiber: IDF refers to non-starch polysaccharides that cannot be dissolved in water or fermented by human gut microbiota, accounting for 2/3 to 3/4 of natural fibers, and are often found in plant roots, stems, trunks, leaves, skins, and fruits [6]. IDF is contained within common diets like enoki mushrooms, carrots, and oats, and the functional properties of different foods vary according to their IDF composition and structure [8]. What's more, IDF absorbs water in the large intestine to soften the feces and increase their volume, thereby preventing constipation [9].

2.2. Sources of dietary fiber

"The scientifically validated study of polymers of novel carbohydrates carried out by the Chinese Society of Nutrition in 2021, through the search and integration of databases and standards, declared that the sources of dietary fiber are of 3 types: naturally occurring in plants; obtained by extracting from plants by physical, chemical, and enzymatic methods; and obtained by synthesis [10]." Natural dietary fiber can be found in a variety of foods, including but not limited to fruits, vegetables, and grains. Dietary fiber-rich foods include wheat bran, citrus fiber, pea fiber, oat fiber, and beet fiber [10]. Cereal is considered a significant dietary fiber source due to its high content of insoluble dietary fiber (IDF). Moreover, cereal-based sources of dietary fiber have been found to have greater efficacy in regulating blood glucose levels when compared to alternative sources. There are 10 types of extracted and synthetic dietary fibers recommended by the Chinese Nutrition Society, which are galactooligosaccharides, fructooligosaccharides, inulin, polydextrose, β -glucans, cellulose, resistant maltodextrin, alginate, guar gum, and pectin [10].

3. Physicochemical and functional properties of dietary fiber

3.1. Physicochemical properties

The structural characteristics of dietary fibers are the basis of their physicochemical properties, which subsequently have a substantial impact on their functional capabilities. It frequently exhibit a flaky or flocculent, with folds and pores on the surface, some with small particles on the surface, and those with a loose structure, many folds, large pores, and low surface roughness, therefore, having better physicochemical properties [11]. Dietary fibers possess several physical and chemical characteristics mainly contain ion exchangeability, hydration qualities, oil holding capacity, adsorption capacity and solubility.

3.1.1. Hydration capacity. Dietary fiber, as a class of polysaccharides, has hydrophilic groups such as hydroxy(-OH) and carboxy(-COOH) in its chemical structure, and because of the physical structure of the particles on its surface, it has strong hydration capacity. The hydration properties of dietary fibers cause them to absorb water and swell when entering the body, increasing their volume and the weight of faeces to promote constant intestinal peristalsis, and shortening intestinal transit time and reducing intestinal exposure to secondary bile acids and other toxins [12]. It helps prevent gastrointestinal diseases such as constipation and rectal cancer.

3.1.2. Absorption. Dietary fiber molecules have a certain length, strength, and elasticity, and in the digestive tract of the body can form a mesh structure with a certain strength and volume, in which the mesh structure has physical adsorption properties [13]. The substance has the ability to bind and adsorb various chemicals, resulting in a deceleration of fat and carbohydrate absorption. Additionally, it promotes the excretion of bile acid, so exhibiting hypoglycemia and hypocholesterolemic effects [14]. In addition, dietary fiber has the ability to absorb certain toxic and detrimental constituents found in food, such as nitrite ions and heavy metals, hence mitigating the associated health risks [15]. Among them, natural dietary fibers have a strong adsorption capacity for fats, which is enhanced when treated, e.g., the adsorption capacity of saturated and unsaturated fats used in the experiments reached 6.53g/g and 3.16g/g, respectively, after ultramicrotomy of soybean skin water IDF [16].

3.1.3. Ion exchange capacity. The ion exchange capacity of dietary fiber refers to the fact that in aqueous solution dietary fiber contains side chain groups like hydroxy(-OH) and carboxy(-COOH) can be reversibly exchanged with certain cations and adsorption of the original cations in the solution, mainly including Cu^{2+} , Pb^{2+} , Cd^{2+} , etc., and preferentially exchanges toxic heavy metal ions, such as Pb^{2+} and Cd^{2+} , so that they can be discharged from the body [17]. Furthermore, it has the capacity to modify the immediate ion concentration, influence the pH level, redox potential, and osmotic pressure within the gastrointestinal system, and provide a buffer milieu that is more favorable for the processes of digestion and absorption [18].

3.1.4. Oil holding capacity. The oil holding capacity (OHC) of a material refers to the amount of oil it can absorb per unit of weight. The determination is made by submerging the sample in surplus oil and subsequently centrifuging the liberated oil [19]. OHC plays a crucial role in food applications, such as preventing fat loss and stabilizing food emulsions during food processing, as well as lowering serum cholesterol levels by absorbing fats and oils from the intestinal tract and excreting them after consumption [20].

3.1.5. Viscosity. Some dietary fibers can form a gelatinous state and are viscous, with the amount of viscosity depending on their chemical structure, with cellulose, lignin, etc., being almost non-viscous, but pectin, gum, agar, etc., usually having a strong viscosity [18]. The viscosity of dietary fibers is influenced by their molecular weight, chemical composition, water retention capacity, particle size, dissolving temperature, processing time and pH [2]. The characteristic of dietary fiber extends beyond

its application as a thickening agent in food preparation. For instance, pectin, a type of natural polysaccharide characterized by its intricate structure, serves as a crucial constituent of plant cell walls. It can also be used as a thickener, emulsifier, stabilizer, and other food additives widely used in the food production industry [21]. Viscose polysaccharides exhibit a beneficial impact on the reduction of blood glucose and blood cholesterol levels, while concurrently enhancing the enzymatic activity inside the digestive tract [22].

3.2. Functional properties

3.2.1. Prevention of cardiovascular disease. After a long period of experimental research by scientific researchers, a large amount of clinical data has proved that elevated serum cholesterol levels are one of the causes of coronary heart disease [18]. Therefore, sufficient consumption of dietary fiber has the potential to decrease the binding and adsorption of cholesterol, descent its absorption by the body, thus lowering blood pressure and lipid levels and preventing cardiovascular diseases. Previous studies have demonstrated that individuals who consume the largest amount of dietary fiber exhibit notably reduced rates of cardiovascular disease morbidity and mortality. These advantageous outcomes can perhaps be attributed to the capacity of dietary fiber to decrease levels of total serum and low-density lipoprotein cholesterol (LDL) [23]. Foreign scholars organized experimental population intake of arabinoxylan, which is common in cereals, analyzed serum cytokines of the experimental personnel and conducted metabolomics and genomics studies on them. The results showed that LDL decreased significantly after dietary fiber intake, and the value of bile acid, which helps to lower cholesterol, increased significantly [24].

3.2.2. Prevention and treatment of type 2 diabetes. Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease in which patients mainly manifest high blood glucose, a relative lack of insulin, insulin resistance, dyslipidemia, etc. If no attention is paid to it and the condition is allowed to deteriorate, it may lead to a variety of complications. Mild symptoms include blurred vision, dizziness, headache, weakness, and difficulty in recovering from wounds; more serious complications, and potentially life-threatening diabetic nephropathy (DN) and cardiovascular disease [25]. Current research suggests that dietary fiber may regulate human blood glucose levels through the following three mechanisms: (1) dietary fiber may regulate human glucose metabolism, by inhibiting the digestion and absorption of sugary foods and accelerating their metabolic rate, etc.; (2) dietary fiber may regulate insulin activity, by improving insulin resistance, regulating the release of insulin, and controlling the expression of key genes, etc.; (3) dietary fiber has the potential to exert an impact on the growth and metabolism of the intestinal microbiota, alter the metabolites produced by the gut microbiota, and regulate the ionic environment and pH levels within the intestines. Consequently, it may assume a regulatory function in the progression of diabetes [26, 27]. Dietary fiber infiltrates the gastrointestinal tract and acquires a thick consistency, impeding the absorption and utilization of glucose and prolonging the time it takes for the stomach to empty, hence ensuring consistent blood glucose levels following meals [28]. A considerable body of research has demonstrated that adequate consumption of dietary fiber has the potential to decrease postprandial blood glucose levels in individuals with diabetes, as well as mitigate the likelihood of developing chronic conditions such as glucose intolerance [29, 30]. Furthermore, insulin is produced via insulin cells in healthy individuals, and its primary role is to facilitate the decomposition of blood glucose and the production of glycogen for storage, while also regulating blood glucose levels within a specific range. However, in individuals with diabetes, the impaired insulin cells are unable to produce an adequate amount of insulin, resulting in reduced glycogen synthesis and impaired glucose storage, ultimately leading to elevated blood glucose levels. But dietary fiber can absorb water and become viscous, slowing down the decomposition and absorption of glycogen, thus controlling blood glucose levels in a safe range, and also increasing the sensitivity of glycogen to stimulate insulin secretion [28]. On the other hand, the microbiota of T2DM patients is characterized by a decrease in the consumption of Short Chain Fatty Acid (SCFA), a decrease in the abundance of SCFA, changes in the intestinal flora,

impairment of the intestinal barrier function, and an increase in the level of plasma Lipopolysaccharide (LPS) [31]. Hence, high-fiber diets, low-carbohydrate diets, and Mediterranean diets, which substitute high-fat, high-sugar, low-dietary-fiber diets by advocating for a substantial consumption of fruits, vegetables, and legumes, have demonstrated the ability to elevate the intestinal concentration of short-chain fatty acids (SCFAs). This, in turn, can contribute to the progression of type 2 diabetes mellitus (T2DM) and enhance overall health [32].

3.2.3. Improvement of intestinal health. The consumption of dietary fiber is of significant importance in the prevention of constipation, alleviation of inflammatory bowel disease, regulation of body weight, and prevention of colon cancer. While the human body is unable to digest and absorb dietary fiber, intestinal bacteria have the ability to deconstruct and ferment it to varying extents, resulting in the production of a significant quantity of SCFAs. SCFA have the ability to hinder the growth of tumors through many pathways. This is the primary way in which dietary fiber helps prevent and treat colorectal cancer [8]. What's more, SCFA is the principal carbon energy source for colonocytes [1]. Meanwhile, SCFA produced by fermentation can prevent metabolic disorders, reduce intestinal permeability, decrease lipopolysaccharide translocation, and ultimately improve intestinal barrier function [7]. Research has indicated that dietary fiber, particularly soluble dietary fiber, possesses prebiotic properties that facilitate the proliferation of advantageous bacteria within the gastrointestinal tract while impeding the replication of pathogenic bacteria. Consequently, this process enhances the intestinal milieu, bolsters the host's immune response, and regulates the body's metabolic processes [33]. Dietary fiber has high WHC and SC, which can soften the stool, increase its volume, accelerate intestinal peristalsis, and promote defecation, which not only prevents constipation, but also reduces the time that carcinogens are in contact with the intestinal wall, thus reducing the incidence of colon cancer [18]. At the same time, dietary fiber can form gel in the gastrointestinal tract, which can not only adsorb cholesterol and reduce its absorption, but also exclude some potential carcinogens, such as polycyclic aromatic hydrocarbons, carcinogenic nitrosamines, etc [34]. This gel is resistant to the mechanical stresses exerted by the stomach and can maintain its physical form in gastric acid [7], and the prolonged retention of food in the stomach elicits activation of the traction receptors located in the stomach wall. This activation subsequently transmits satiety signals to the brain, leading to a sensation of satiety and a diminished inclination to consume further food [34], reducing total food intake and thus controlling body weight to prevent obesity. Within the colon, certain fermentable fibers have the capacity to facilitate the proliferation of advantageous bacteria, such as *Lactobacillus* and *Bifidobacterium* [35], while concurrently impeding the replication of detrimental bacteria, which balances gut flora. The development of colon cancer is mainly related to the prolonged residence time of carcinogens in the intestinal tract and prolonged contact with the intestinal wall [36]. Dietary fiber reduces the duration of contact between carcinogens and the intestinal wall, thus reducing the incidence of colon cancer [18]. A lack of dietary fiber in the intestinal flora can increase susceptibility to disease due to a decrease in protective mucus [37].

4. Dietary fiber extraction

The significance of dietary fiber has led to the emergence of a substantial and promising market for foods and components that are rich in fiber. Various methods, including chemical, mechanical, thermal, enzymatic, etc., have been used to extract dietary fiber in recent years. Different extraction techniques have their own advantages and disadvantages, and the dietary fiber produced by different extraction methods will have differences in molecular structure and functional properties.

4.1. Chemical method

The acid and alkaline procedures are the generally employed techniques for chemically extracting dietary fiber. However, using either approach alone necessitates a high concentration of solution, a longer duration, and results in low purity and color of the extracted dietary fiber [6], and a low cost. But the chemical reagents used in the extraction process are generally strong acids and bases, and the

processing temperature is extremely high, resulting in serious corrosion of the containers, pipelines, and material pumps required for extraction. A large number of anions and cations are brought into the experiments, which results in a low content of dietary fiber extracted and poor quality [8]. The main acid reagents used in the current study were citric acid and hydrochloric acid, while the main alkali reagents were sodium hydroxide and sodium carbonate [6]. The addition of hydrogen peroxide during extraction [38]. This facilitates the dissociation of the intimately interconnected components of DF and non-DF, thereby revealing the internal hydroxyl chains and consequently enhancing the water retention ability of DF [6].

4.2. Physical method

The primary physical technologies involved are microwave, ultrasonic, ultra-micro pulverization, extrusion cooking, high-pressure homogenization, and subcritical water extraction. Because the microwave method is not suitable for large-scale production in factories, and the ultrasonic method destroys the cell wall of the plant to be extracted to make the solvent easier to enter, both methods are more suitable as an auxiliary method [39]. There are two general types of ultra-micro pulverization methods: one is dry ultra-micro pulverization and the other is wet ultra-micro pulverization. Extrusion cooking is a continuous process combining high shear, high temperature, and high pressure and can also modify dietary fiber [6]. Various physical approaches have the capability to modify the microcrystalline structure of IDF, thereby augmenting the surface area of particles. Consequently, these methods can induce alterations in the processing functional properties of IDF, including its hydration capacity. Consequently, these modifications have an impact on the physiological and functional properties of IDF. They also have the advantages of relatively simple operation and environmental protection, but compared with other methods, their equipment cost is relatively high, and there are still some limitations in industrial production [40].

4.3. Biological method

The fundamental biological approaches encompass enzymatic and fermentation techniques. The enzymatic method is the use of specific enzymes for the specific degradation of non-dietary fiber components, such as α -amylase, which can degrade starch, protease, which can degrade proteins, etc. The advantages of the enzyme approach include its great efficiency and lack of contamination, but there are also the disadvantages of poor controllability [8]. Enzymes have the advantages of specialization and specificity; usually, the use of the composite enzyme method will result in a higher DF extraction rate and purity of extraction than the single enzyme [6]. Fermentation is the use of microbial output of proteases and amylases to degrade the non-dietary fiber components of the material, which allows for the extraction of DF [6]. The commonly used fermentation microorganisms include *Aspergillus Niger*, cellulase, *Lactobacillus*, *Lactobacillus bulgaricus*, green wood mould, *Streptococcus thermophilus*, and others.

4.4. Combined extraction method

The combined extraction method refers to the extraction of dietary fiber obtained by combining two or more of the above extraction methods, such as the enzyme and chemical combination method, the ultrasound-assisted method, the microwave-assisted method, etc., which can combine the advantages of multiple methods, improve the deficiencies of using a single method, enhance the beneficial characteristics of dietary fiber [40]. Since both physical and enzymatic methods have the advantages of higher extraction rates, relatively simple production operations, good properties of the IDF obtained, and environmental friendliness, combining these two methods not only combines the advantages of the two methods but also reduces the costs associated with a single method and has a better application prospect [40]. Peng et al. demonstrated that the combined microwave and enzyme treatment could release higher levels of phenolic acids compared with the treatment alone, and the obtained grapefruit peel IDF showed stronger antioxidant activity [41]. Therefore, the combined extraction method is

relatively simple to operate, reduces cost, saves energy, and produces high yields and high-quality dietary fiber [40].

5. Conclusion

Dietary fiber, due to its own physical and chemical properties, is effective in controlling blood glucose, blood pressure, reducing appetite and body weight, and also reduces the level of serum cholesterol absorbed by the body. Moreover, dietary fiber has been found to be highly efficacious in the prevention and alleviation of types 2 diabetes, rectal cancer, inflammation, and constipation among patients. While the human body is unable to digest and absorb dietary fiber, it can undergo fermentation by specific intestinal microbiota in the large intestine. The primary outcome of this fermentation process is the production of short-chain fatty acids (SCFA). SCFA serves various functions such as supplying energy to human intestinal cells, regulating energy metabolism, glycolipid metabolism, and intestinal flora. Additionally, SCFA aids in maintaining intestinal barriers and health, as well as enhancing intestinal immunity. In addition to protecting the digestive system's ability to function, the breakdown of dietary fiber in the colon encourages the growth of good bacteria and suppresses the growth of harmful bacteria. High-quality dietary fiber has been continuously extracted from vegetables, fruits and legumes using enzymatic, fermentation and ultrasound-assisted processing.

Dietary fiber, as one of the seven essential nutrients for human health, has an important research value and a wide range of application prospects. People can continue to explore and study the high dietary fiber-rich grains in nature, their extraction and modification, and at the same time, people can develop new extraction processes or continuously optimize the extraction and processing methods of dietary fiber, and apply it more to food processing and functional foods. Subsequent studies can also continue to delve deeper into the study of dietary fiber in the gastric and large intestinal environments, as well as the role it plays in the small intestine.

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The relationship between vitamin D and periodontal disease

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Abstract. In recent years, periodontal disease has been plaguing many people, and the topic of this study is the relationship between periodontal disease and Vitamin D. Aspects are divided into Vitamin D and periodontitis, which are discussed separately. The research question is mainly in the discussion of the disease, and experiments are conducted using simulation tests to draw conclusions to do the control and observation methods to draw conclusions to take samples, there are also based on people's daily habits to make reference to improve the treatment methods and the results of the study. The conclusion is that Vitamin D and dental care are inseparable; the lack of trace elements will lead to a decline in resistance and ultimately the formation of periodontitis. Vitamin D is the primary factor affecting the growth and protection of bones, so Vitamin D will make the teeth stronger.

Keywords: Vitamin D, Periodontal Disease, Dental Health

1. Introduction

Periodontal disease is a disease that occurs in the supporting tissues of the teeth, including two major types of gum disease: periodontitis, which only involves the gum tissues, and periodontitis, which affects the deeper periodontal tissues. Periodontal disease is a common oral disease, one of the main causes of tooth loss in adults, and a major oral disease that endangers human teeth and general health. Vitamin D (VD for short) is a fat-soluble Vitamin function that can prevent rickets, promote skin cell growth and differentiation, and regulate immune function. In general, frequent exposure to sunlight does not cause deficiency disease in adults, infants, pregnant women, lactating mothers, or infrequent outdoor activities in the elderly to increase the supply of Vitamin D to 10 µg per day. Vitamin D deficiency can increase the risk of the organism developing Vitamin D deficiency can increase the risk of periodontitis, and Vitamin D is also involved in the progression and treatment of periodontal disease. Inadequate levels of Vitamin D may accelerate the progression of periodontal disease, and supplementation of Vitamin D in appropriate amounts can promote the maintenance of periodontal health, improve the efficacy of periodontal therapy, and inhibit the resorption of alveolar bone, among other things. The relationship between Vitamin D and periodontitis is characterized by its classical role in the regulation of bone metabolism and its immunomodulatory role in the inflammatory process.

This article provides a review of the progress of research on Vitamin D and its relationship with periodontitis.

2. Analysis

It is important to understand that healthy periodontal tissue is more likely to be achieved with adequate serum Vitamin D levels. Adequate serum Vitamin D levels in the treatment of periodontal disease not only reduce the incidence of tooth loss and periodontitis, but also promote periodontal tissue healing. Vitamin D supplementation is effective in maintaining periodontal health, especially in improving bleeding on probing, gingival index, and probing depth, increasing bone density in the jaws, and inhibiting alveolar bone resorption. The effect of Vitamin D on the development of periodontitis and its severity may be due to its regulatory effect on bone calcium metabolism and its inhibitory effect on periodontal pathogenic bacteria and inflammatory transmitters involved in periodontal destruction [1]. Vitamin D improves intestinal absorption of calcium. As calcium from food passes through the digestive system and enters the circulation, Vitamin D promotes the absorption and translocation of calcium into the bones and teeth. This enhanced calcium absorption helps maintain healthy bones and teeth. Vitamin D helps maintain calcium balance in the body. It ensures proper levels of calcium by regulating the excretion of calcium by the kidneys and the absorption of calcium by the intestines. This is important for maintaining healthy teeth because calcium in the teeth is a key component of dentin and enamel. The alveolar bone is an important tissue that supports the teeth and Vitamin D is vital to its health. Vitamin D deficiency may lead to thinning of the alveolar bone and osteoporosis. Vitamin D strengthens the alveolar bone by promoting calcium absorption and bone formation. Healthy alveolar bone provides good support, which keeps teeth stable and healthy. Vitamin D has anti-microbial activity and can exert an inhibitory effect on many oral pathogenic bacteria. Studies have shown that Vitamin D can inhibit a number of common oral pathogenic bacteria, such as *Actinomyces* and *Streptococcus gingivalis*. This anti-microbial action helps to reduce the number and activity of pathogenic bacteria in the mouth, thereby reducing the risk of oral infections and diseases. Vitamin D plays an important role in regulating the immune system by modulating the immune response of the oral mucosa [2]. Moderate levels of Vitamin D help enhance the immune function of the oral mucosa, increasing resistance and reducing the risk of infection. In addition, Vitamin D can reduce symptoms such as oral inflammation and bleeding gums. Vitamin D plays an important role in the balance of the oral microbiota. It helps regulate the balance between beneficial and pathogenic bacteria. Vitamin D increases the abundance of beneficial bacteria, such as lactobacilli, while reducing the number of pathogenic bacteria. This balance is important for maintaining oral health, as too many pathogenic bacteria can lead to oral problems such as tooth decay and periodontal disease. Vitamin D's immunomodulatory and anti-microbial effects give it the potential to prevent and treat dental caries and periodontal disease. Proper Vitamin D levels help reduce the incidence and progression of dental caries and help control the inflammatory response to periodontal disease.

Then do the controls. Several case-control studies have found a positive correlation between Vitamin D deficiency and periodontitis. These studies reached their conclusions by comparing Vitamin D levels between individuals with periodontitis and healthy controls. The results showed that people with Vitamin D deficiency were at a higher risk for periodontitis [3]. Several longitudinal cohort studies also support the association between Vitamin D deficiency and periodontitis. These studies followed a large number of participants for many years and assessed their Vitamin D levels and the onset of periodontitis. The results showed a significant association between Vitamin D deficiency and an increased risk of subsequently developing periodontitis. Vitamin D deficiency may affect the development of periodontitis through several mechanisms. Firstly, Vitamin D deficiency affects the function of the immune system, making the oral mucosa less resistant to pathogenic bacteria. Second, Vitamin D deficiency may lead to the destruction of the alveolar bone and the formation of periodontal pockets, thus providing conditions for the development of periodontitis. It is important to note that although some correlation exists, the causal relationship between Vitamin D deficiency and periodontitis has not been fully clarified. Other factors, such as oral hygiene status and dietary habits, may also influence the risk of periodontitis. Therefore, caution is needed in interpreting these epidemiological results and further more in-depth studies are needed to confirm the relationship and understand the mechanisms involved. In the meantime, maintaining proper oral hygiene and a balanced diet are also important measures to

prevent periodontitis. Vitamin D has an important immunomodulatory function and can influence the immune response of the oral mucosa. In the pathogenesis of periodontal disease, the abnormal reaction of the immune system and inflammation play a key role. Vitamin D deficiency may lead to immune system dysfunction, making the oral mucosa less resistant to pathogenic bacteria, thus promoting the development of periodontal disease. Vitamin D and calcium are closely related, and Vitamin D deficiency affects calcium absorption and metabolism. Periodontal disease is often accompanied by loss and destruction of alveolar bone, which may be exacerbated by Vitamin D deficiency. An imbalance in calcium balance caused by Vitamin D deficiency may lead to thinning of the alveolar bone and damage to the periodontal tissues, thus increasing the risk of periodontal disease. Vitamin D also has anti-inflammatory properties that modulate the inflammatory response. Periodontal disease is a chronic inflammatory condition, and Vitamin D deficiency may lead to overactivation of the immune system and an increased inflammatory response. This inflammatory state can destroy periodontal tissue and further promote the progression of periodontal disease. Despite these potential causal links, the exact causal relationship still needs to be confirmed by more in-depth studies. Other factors, such as genes, oral hygiene and dietary habits, can also play an important role in the development of periodontal disease. Therefore, when assessing the relationship between Vitamin D and periodontal disease, multiple factors need to be considered and more comprehensive, long-term studies need to be conducted to further reveal the mechanism of this relationship. In the meantime, maintaining appropriate Vitamin D intake, good oral hygiene practices and a healthy lifestyle are all important measures to prevent and control periodontal disease [4]. It affects the function and differentiation of immune cells and regulates the inflammatory response. Vitamin D deficiency leads to dysfunction of the immune system, including reduced resistance to oral microbes. This means that people with Vitamin D deficiency are more susceptible to pathogenic bacteria in the mouth, which increases the risk of gingivitis and periodontitis. Vitamin D greatly affects bone health. Vitamin D deficiency affects calcium balance, which in turn leads to lower bone mineral levels and osteoporosis. During the development of periodontal disease, destruction and loss of alveolar bone are common occurrences. Vitamin D deficiency may exacerbate this, further compromising the stability of periodontal tissues and leading to the development of periodontal disease. Periodontal disease is a disease caused by a chronic inflammatory response, and Vitamin D has anti-inflammatory properties. Vitamin D deficiency leads to an over-activation of the immune system and an increase in the inflammatory response, which can exacerbate the severity of periodontal inflammation. A persistent inflammatory state causes damage to the gums and periodontal tissues and promotes the progression of periodontal disease. These biological mechanisms do not exist in isolation, but are interrelated and influential.

The research from Khademi, Z. and Hamed-Shahraki, S. divided a randomized controlled trial of participants with chronic periodontitis into two groups. One group received daily Vitamin D supplements for six consecutive months, while the other group received a placebo. Results showed significant improvements in gingival inflammation and clinical attachment levels in the Vitamin D group compared to the placebo group. A double-blind study investigated the effects of high-dose Vitamin D supplementation on periodontal health in postmenopausal women with osteoporosis. After one year of supplementation, the researchers observed a reduction in pocket depth and an improvement in bone density in the Vitamin D group, which was significantly different from the placebo group. A systematic review and meta-analysis examined multiple clinical trials to assess the overall impact of Vitamin D supplementation on periodontal health. The results of the analysis showed an association between Vitamin D supplementation and reduced levels of gingival inflammation and improved clinical attachment levels in patients with periodontal disease. Potential Benefits of Vitamin D Supplementation as an Adjunctive Therapy for Periodontal Disease: Vitamin D has anti-inflammatory properties that may help reduce gingival inflammation associated with periodontal disease and provide relief. By modulating the immune response, Vitamin D may help maintain a healthier periodontal environment. Vitamin D plays an important role in immune function, promoting the body's ability to fight infection and maintain oral health [5]. Supplementation with Vitamin D may enhance the immune system's defence against periodontal pathogens. Vitamin D is essential for calcium absorption and bone

metabolism. Vitamin D supplementation may help maintain proper bone density and support the integrity of the alveolar bone needed for periodontal disease. Optimal Dose, Duration, and Method of Administration of Vitamin D Supplementation for Periodontal Disease: The optimal dose has not been determined, but general studies have used daily supplementation of 800 to 5,000 international units (IU). The appropriate dose may depend on individual factors such as age, overall health, and Vitamin D levels. Regular monitoring of Vitamin D levels can help determine the appropriate dose for each individual. The duration of clinical trials evaluating the effects of Vitamin D supplementation on periodontal health has ranged from three months to one year.

Finally, although clinical trials have shown the potential benefits of Vitamin D supplementation for periodontal health, the mechanisms of action are not yet fully understood [6]. More in-depth studies could explore the biological effects and molecular mechanisms of Vitamin D in periodontal tissue. Current research is still limited to small-sample clinical trials, and multi-centre randomised controlled trials are needed to validate and strengthen existing findings. This will ensure reliability of data and applicability for replication. The duration of most studies is short, and long-term effects and durability remain unclear. Long-term follow-up studies could assess the lasting effects of Vitamin D supplementation on periodontal health. In order to arrive at more definitive recommendations, larger, well-designed clinical studies are needed to: Determine the dose-response relationship between Vitamin D supplementation dosage and periodontal health to determine the optimal dosage range. Examining differences in response to Vitamin D supplementation across age, gender, race, and severity of periodontal disease. Conduct long-term follow-up studies to assess the lasting effects of Vitamin D supplementation on periodontal health and to understand potential side effects and safety. To address Vitamin D deficiency and promote periodontal health, the following public health strategies could be explored and implemented: Provide information on the importance of Vitamin D, its sources, and methods of supplementation to increase public awareness. This can be achieved through social media, brochures and collaboration with health professional organisations. Increase the amount of Vitamin D in food, e.g., through food fortification programmes, adding Vitamin D to milk, fish, and cereals. Pay special attention to populations that are vulnerable to Vitamin D deficiency, such as older people, pregnant women, indoor workers, and people with dark skin. Provide regular testing and advice on supplementation. Encourage people to spend more time outdoors and receive adequate sunlight so that the body can synthesise enough Vitamin D on its own. Healthcare providers can develop guidelines to provide accurate advice on Vitamin D supplementation and incorporate it into oral healthcare programmes.

3. Conclusion

Overall, it affects the connective tissue around the teeth and the alveolar bone that supports them. Vitamin D is an important fat-soluble Vitamin that is essential for bone health and immune system function. Vitamin D plays an important regulatory role in the immune system. Vitamin D inhibits the production of inflammatory factors and promotes the activity of anti-inflammatory cells. It also contributes to calcium absorption and bone formation and maintenance. Vitamin D's potential role in the prevention and management of periodontal disease is summarised as follows: Vitamin D inhibits the onset and progression of inflammatory responses. Vitamin D's modulatory effects on the immune system help to balance the immune response and reduce excessive immune activity. Vitamin D regulates the immune system to help balance the immune response and reduce excessive immune activity and inflammation, and Vitamin D promotes calcium absorption and bone health. In periodontal disease, Vitamin D deficiency may lead to a reduction in the amount of bone in the supporting tissues of the teeth and an increase in alveolar bone resorption, and Vitamin D enhances the production of antimicrobial peptides, which are known to act against oral pathogenic microorganisms. This paper calls for further research and collaboration on more multi-centre, randomised controlled trials to investigate the effects of Vitamin D on periodontal health.

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Study on bacteriostatic activity of bacillus against penicillium extensus and its application to pear

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Abstract. Currently, research has shown that *Bacillus* plays a crucial role in preserving, storing, and transporting fruit and vegetable products, leading to positive economic outcomes. Through the discussion results, it was found that *Bacillus polymyxoid* HT16 had a remarkable inhibitory effect on *Streptospora nigrospora* of pear. The observations made using scanning electron microscope and it revealed that the spores of the pathogenic bacteria barely germinated after the treatment of crude protein extraction. Additionally, there was a noticeable ablation phenomenon, with the mycelium appearing twisted and atrophied. Furthermore, the outer epidermis of the mycelium was shed and broken. Similarly, the bacteria used for biocontrol also demonstrated a significant impact on suppressing harmful bacteria in grape plants, contributing to the ongoing research on the mechanisms behind grape biocontrol. Providing a theoretical basis for the application of *Bacillus amyloolytica* 22T in pear biological control, the antibacterial activity against *penicillium extensus* was evaluated. This passage provides a review of the inhibitory activity of bacillus against penicillin expansus.

Keywords: *Bacillus*, Fermentation broth, *Penicillium elongatum*, Pear, Biological control

1. Introduction

Pear is known for its natural geographical distribution and economic value. It has the remarkable ability to clear heat and detoxify, promote fluid and quench thirst, and replenish water in the body. The pear is packed with an abundance of fruits, microorganisms, and an array of trace elements. Nevertheless, the issue of pear mold can pose a significant challenge during transportation. There are various factors that can contribute to pear mildew, such as black heart, fruit stem rot, *penicillium*, black skin, brown spot, black spot, and more. Similar to a biochemist, the storage period of pear fruit is greatly impacted by pathogenic bacteria. This disease during the storage process significantly affects the quality and duration of storage, leading to a significant reduction in economic value.

Conventional methods of food sterilization also come with their own limitations. Thus, current research is centered around biological sterilization. The study revealed the significant impact of *Bacillus* on the prevention of corrosion in fruit and vegetable products during transportation, storage, and preservation [1]. Additionally, it plays a crucial role in enhancing the economic value of these products. Typically, *Bacillus* refers to the genus *Bacillus* of the *Bacillus* family, which relies on oxygen to produce spores. It is found in various places in nature and can persist for long periods of time in the form of spores. Currently, *Bacillus* is primarily used to combat biological spoilage caused by grey mold and *penicillium*. Due to the prevalence of *Bacillus* in nature, one of their remarkable characteristics is their

ability to produce spores that are highly resistant to stress. This makes *Bacillus* a valuable tool in production practices for preventing and controlling fungal and bacterial diseases in fruits, vegetables, crops, and more. *Bacillus subtilis* has proven to be effective in preventing peach brown disease and litchi fruit rot. The inhibitory effect of *Bacillus polymyxoides* HT16 on postharvest black spot of pear also demonstrated significant potential for application. This paper thoroughly examines the inhibitory activity of *Bacillus* on penicillium of pear and other related studies. It also includes references and offers suggestions on how to effectively apply bacillus to prevent spoilage during the transportation of agricultural products.

2. Overview

The term “*Bacillus*” typically denotes the genus *Bacillus* within the *Bacillus* family, which is characterized by its ability to produce spores of gram-staining positive bacillus. This bacterium is widely spread in the natural environment and can persist in the form of spores for extended periods of time. Currently, *Bacillus* is mostly employed for the purpose of preventing and managing biological deterioration in griseus and penicillium. As an illustration, the inhibitory effects of *Bacillus subtilis* H110 from Qidongplum on postharvest penicillium and black spot of apple pear were observed [2]. Numerous studies have demonstrated the efficacy of diverse *Bacillus* H110 treatments in mitigating the detrimental impacts of penicillium and black spot on apple pear fruit. *Bacillus* is a significant microbial group that is abundant in nature. Its notable characteristic is its ability to generate highly resistant spores. This makes *Bacillus* suitable for use in production practices to prevent and manage fungal and bacterial diseases in fruits, vegetables, crops, and other crops.

2.1. *Bacillus* and its application in agriculture

One potential application of *Bacillus subtilis* is its utilization in the prevention and management of peach brown disease and litchi fruit rot. One of the bacilli, *Bacillus polymyxoides* HT16, has demonstrated significant potential in the inhibition of postharvest black spot in pear. The assessment of the application efficacy of *Bacillus polymyxoides* HT16 on the pear black spot pathogen revealed a highly potent inhibitory impact, with an inhibitory rate of 53.2% [3]. The findings of the study indicate that *Bacillus polymyxoides* HT16 exhibited a noteworthy inhibitory impact on *Streptospora nigrospora* strains found in pear. The utilization of scanning electron microscopy and electron microscope revealed that following the application of crude protein extraction treatment, the germination of pathogenic bacterial spores was minimal. Additionally, an ablation phenomena was seen, accompanied by the twisting and atrophy of mycelia, as well as the detachment and breaking of the outer epidermis of the mycelia. The findings of this study demonstrate the viability of *Bacillus polymyxoides* HT16 as a bacterium for biocontrol purposes. This study aimed to explore the effects of *Bacillus amylus* 22T on colony diameter, spore germination rate, and mycelium content of *Penicillium* expansions, using the prior configuration of LB liquid medium, LB solid medium, and PDA plate medium. For the purpose of assessing the antibacterial efficacy of *Bacillus amylolytica* 22T against *Penicillium extensus*. Furthermore, it aims to establish the theoretical foundation for its implementation in the biological management of pear.

2.2. *To expand the harm of penicillium in pear and its control measures*

The fruit possesses a high nutritional content and an ample water content, thereby establishing a conducive environment for the proliferation of microorganisms, including mold. Once subjected to microbial infection, it becomes susceptible to mildew. *Penicillium* typically occurs subsequent to fruit mildew. *Rhizopus*, *mucor*, *Aspergillus*, and *penicillium* are among the commonly encountered molds. Typically, mold infiltrates the fruit following damage, proliferates within the fruit, and leads to mold growth and decay. The microorganisms create toxins, which are harmful metabolic compounds, during their growth, which can negatively impact human health. Picillin is one of these poisons. Moldy fruits and their derivatives, such as apples, pears, hawthorns, tomatoes, and apple juice, are the primary sources of this substance. Penicillins, which are the predominant toxin found in moldy fruits, are

synthesized by *penicillium diffusum*. Penicillin, a toxic substance, possesses detrimental effects on the gastrointestinal system, as well as exhibiting carcinogenic and teratogenic characteristics. Aflatoxin is the most poisonous. Aflatoxin is widely recognized as the most carcinogenic toxin identified to date. Spoiled peanuts, corn, and dairy products are sources of this substance. Additionally, it can be observed in mildewed raisins and various other dehydrated fruits. Aflatoxin primarily elicits the development of liver cancer and subcutaneous sarcoma, exhibiting a carcinogenic impact that is 75 times higher than that of hazardous substances such as dimethylnitrosamines. Certain types of molds have the potential to induce renal and hepatic impairment, as well as neurological diseases. The presence of mildew in fruit can lead to financial losses during the processes of transportation and storage.

Current research suggests that *Bacillus* exhibits significant promise as a biocontrol agent and a means of preventing some postharvest illnesses affecting fruits. For instance, the research and analysis conducted by Che Jianmei demonstrated that *Bacillus* SPP exhibits the most effective inhibitory impact on saprophytic bacteria and fungi in the Longan region [4]. *Bacillus licheniformis* W10, a recently identified biocontrol bacterium, has the ability to metabolize antibacterial proteins. The research conducted by Ling Zheng reveals that the bacteria and antimicrobial proteins present during the fruit storage period of apple ring spot (*Botryosphaeria berengeriana* f.), anthrax bacteria (*Glomerella Coagulate*), and citrus *Penicillium* germs (*Penicillium Citrinum*) exhibit antagonistic effects [5]. This bacterium demonstrates a favorable preventive effect on postharvest ring spot, anthracnose, and *penicillium* in both apple and citrus fruits. The findings indicated that the W10 bacterial solution, culture filtrate, and antibacterial protein had significant detrimental and inhibitory impacts on the mycelial shape and proliferation of the three pathogens. The compound W10 exhibited the most pronounced impact on the mycelia of the fungus. The presence of W10 was found to exert an influence on the processes of sporulation, spore germination, and bud tube formation in the bacteria. In conclusion, *Bacillus* exhibits significant promise for the preservation of fruit.

2.3. A review on the antibacterial activity of *Bacillus* against *Penicillium expansum*

The results indicate that *Bacillus* has a significant impact on the prevention and control of spoilage in the majority of fruits and vegetables. An experiment conducted by Du Hongyan demonstrated the impact of *Bacillus amylolityca* on the prevention and control of grape powdery mildew. The results indicated that *Bacillus amylolityca* was successful in effectively managing grape powdery mildew and enhancing photosynthesis in grape leaves [6]. The data is presented in the following manner: During the initial peak period of grape powdery mildew, the control effect of *Bacillus amylolityca* was found to be significantly lower than that of fungicides ($P < 0.05$) after the third application. However, it still achieved a control rate of 70.00%. In the second peak of grape powdery mildew, after three applications, the control effect of *Bacillus amylolityca* was 65.00%, which was significantly higher than that of the fungicide ($P < 0.05$). Thus, *Bacillus amylolityca* has the potential to serve as an effective method for managing grape powdery mildew in Yunnan's agricultural regions. The study conducted by Zhang Lili investigated the indoor inhibition effect of *Bacillus subtilis* on pear fruit rot prevention and control. The results indicated that the culture of *Bacillus subtilis* Sf-19 exhibited the most effective comprehensive inhibition effect. It achieved a plate inhibition rate of 87.33% for mycelium growth and spore germination inhibition rate. The study on the inhibition effect of *fructus verticillaris* on fruit was conducted in two parts: protection (I) and treatment (II). The protection test (I) of the Sf-19 cultured stock solution resulted in an inhibition rate of 71.97% for pear *verticillaris*. Similarly, the treatment test (II) showed an inhibition rate of 64.92%. The inhibitory effect of the Sf-28 culture solution on pear ring fungus was evaluated comprehensively. The plate inhibition rate of mycelia growth of pear ring fungus was found to be 87%, while the inhibition rate of spore germination was 80%. In the fruit pear ring fungus protection test (I), the Sf-28 culture solution exhibited an inhibition rate of 64.54%. Similarly, in the treatment test (II), the inhibition rate was measured to be 59.21%. The test conducted yielded a significant effect, with an observed inhibition rate of approximately 40% on pear mold. In conclusion, it can be observed that the practical implementation of *Bacillus* for biological control is supported by a strong theoretical basis.

Additionally, the utilization of bacillus for the control of corrosion in vegetables and fruits exhibits significant potential.

3. Review of relevant Experimental results

3.1. *Penicillium expansum*

The colonies of *Penicillium expansum* exhibit small blotchy, grassy green appearance with a cinnamon-colored back and small red spots in the center. The tip of the conidial peduncle consists of 1-2 brush-like branches, while the peduncle itself has a slender, bottle-shaped structure. The conidia exhibit an oblate or round shape and possess a greenish coloration when they are clustered together. The conidia are characterized by their small size, measuring $1.8 - 2.2 \times 1.8 - 2.2 \mu\text{m}$, as depicted in figure 1.

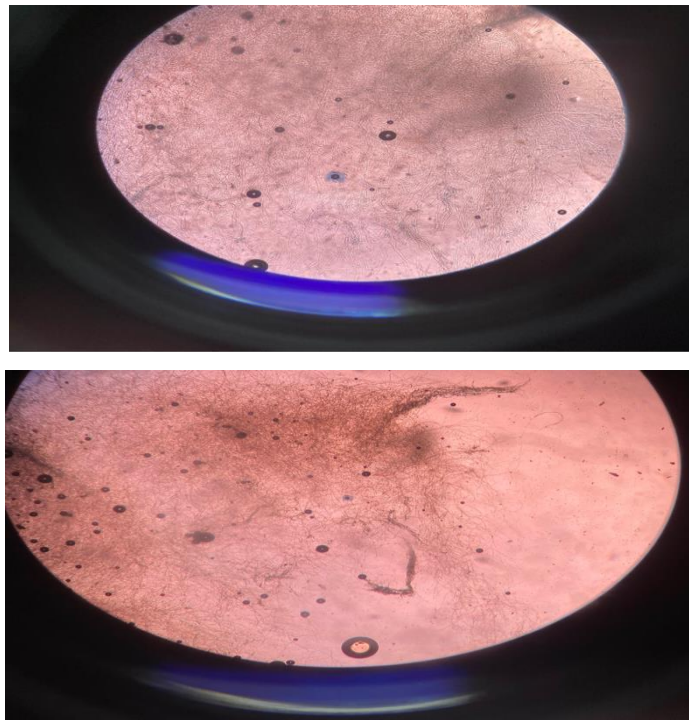


Figure 1. Mycelium imaged under a microscope from Zhejiang University

3.2. *In vivo inhibition test*

The strain *Bacillus* 22T exhibits significant potential for the biological control of pears. The experimental data, depicted in Figure 2, reveals that the average incidence of pear fruit treated with 22T within a 48-hour timeframe is 66.6%. This percentage is significantly lower compared to the incidence observed in pear fruit treated with normal saline. The average bacteriostatic zone of the 22T fermentation solution was measured to be 0.5 units.

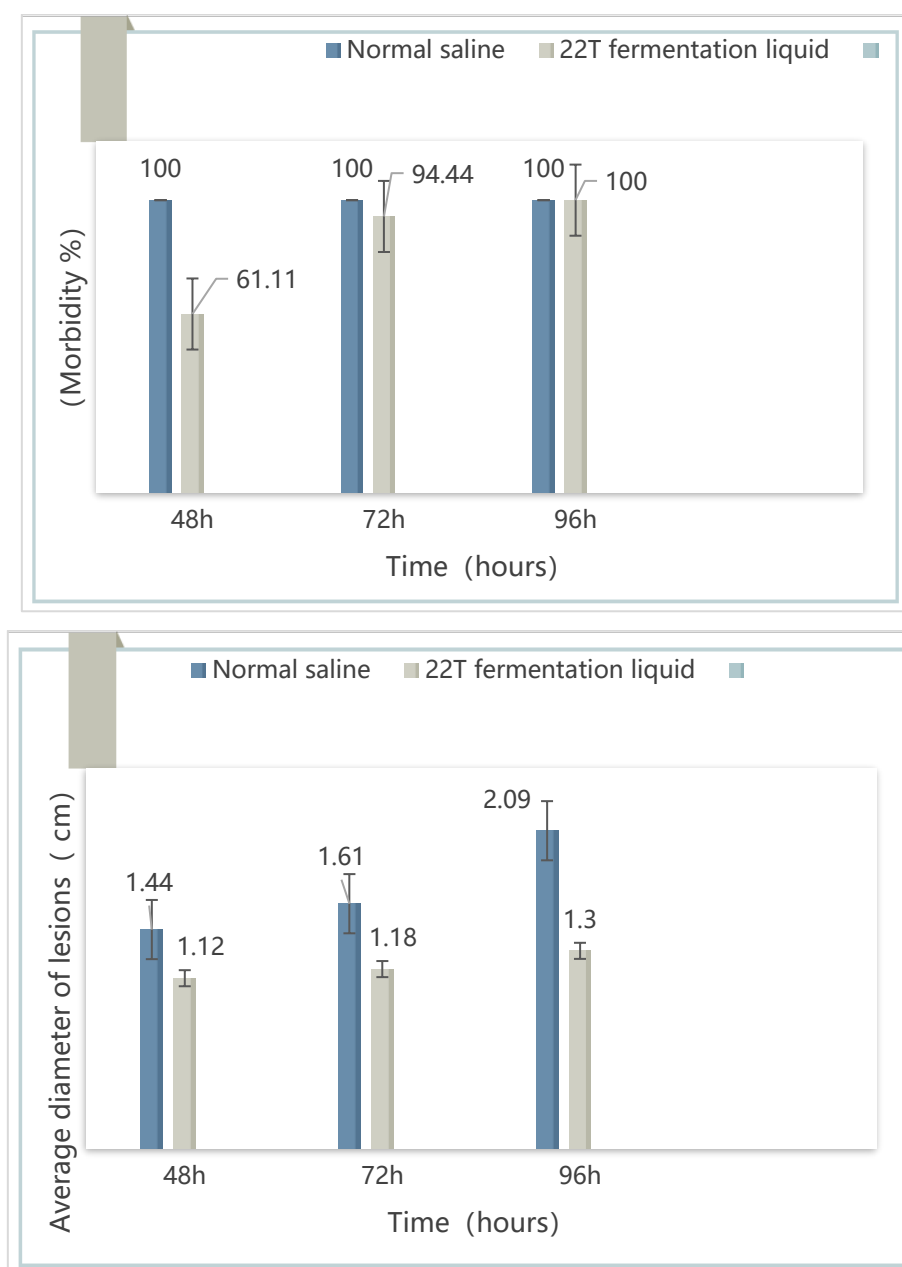


Figure 2. Inhibitory effect of Bacillus 22T on Penicillium

4. Conclusion

The bacteriostatic effect of Bacillus 22T on pear fruit is readily apparent. The application of 22T has a clear and noticeable impact on the eradication of penicillium extensus, making it a promising candidate for biocontrol against pear rot disease. The potential of Bacillus 22T in preventing and controlling pear penicillium disease is promising. Additionally, it has demonstrated a notable ability to inhibit pathogenic bacteria. Therefore, there is a possibility for its practical application. However, this paper is deficient in comprehensive research and fails to engage in thorough investigation, providing only superficial experimental results. Thus, it is recommended to postpone this experiment until the conditions have been further enhanced and stabilized in order to conduct a more comprehensive study, enhance the accuracy of the results, and obtain more precise experimental reports and data.

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Characteristics and neural mechanisms of active forgetting in patients with PTSD

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Abstract. The intrusive thoughts and memory function disorders caused by PTSD have a negative impact on the lives of patients, and the neural mechanism of active amnesia is not yet fully understood. This study aims to explore the characteristics and neural mechanisms of active forgetting in PTSD patients. By studying the characteristics of directed forgetting and suppression-induced forgetting in PTSD patients, as well as understanding and analysing relevant paradigms, behavioural evidence and neural evidence, we finally found that the mechanism of directed forgetting and suppression-induced forgetting in PTSD patients are different. That is, the directional amnesia effect of patients with high-level PTSD is higher than that of patients with low-level PTSD, while the suppression-induced forgetting effect of patients with high-level PTSD is lower than that of patients with low-level PTSD. The reason is that patients with high-level PTSD have a strong sense of self-protection, so they actively avoid emotional materials during the encoding stage of memory, resulting in selective encoding of emotional materials. Therefore, the directed forgetting effect is strong. Individuals with high levels of PTSD have a weakened ability to inhibit emotional material, leading to their inability to stop involuntarily ruminating on it, and thus their suppression-induced forgetting effect is weak. Overall, this study understands the key factors that are beneficial to the recovery of PTSD patients, and at the same time provides certain reference and guidance for the treatment plan of PTSD patients.

Keywords: PTSD, active forgetting, neural mechanism

1. Introduction

PTSD (post-traumatic stress disorder) refers to a mental disorder that results in delayed onset and long-term persistence after experiencing unusually threatening or catastrophic stressful events or situations [1]. It is characterized by disorders of memory and forgetting functions [2], and unconscious or unconscious intrusion of traumatic memories [3]. This has a great impact on the mental health and life of PTSD patients, such as anxiety, work and interpersonal problems. Recent research has begun to focus on the neural mechanisms of active forgetting in PTSD, mainly studying how individuals forget traumatic experiences. Active forgetting refers to the phenomenon of increased forgetting caused by individuals consciously inhibiting the recall of certain memory contents [5], which can occur in the encoding stage and retrieval stage of memory [6]. It helps solve the symptoms of PTSD patients such as continuous, involuntary recurrences and flashbacks of traumatic events. It can also serve as a self-protection mechanism for PTSD patients and help them alleviate their psychological disorders. However,

the cognitive neural mechanisms of active forgetting are not yet fully understood. Therefore, this study explored the neural mechanism of active forgetting in PTSD patients when faced with traumatic memories.

The main purpose of this study is to describe the cognitive neural mechanism of active forgetting in PTSD patients by analysing experimental behavioural results and neurophysiological mechanisms in the existing literature. And by understanding the physiological and neural mechanisms of PTSD patients to help them regulate traumatic memories. At the same time, this study will also explore the activation areas of the brain during the active forgetting process and the interactions between brain areas to build a more comprehensive explanatory framework for the active forgetting mechanism of PTSD patients, to more deeply explore how PTSD patients pass through Active forgetting modulates traumatic memories.

Theoretically, this study helps to reveal the key factors in the recovery process of PTSD patients and can also better construct the theory of PTSD patients in coping with traumatic memories by in-depth understanding of the cognitive neural mechanisms of patients' active forgetting. Clinically, the significance of this study is to provide psychotherapeutic workers with specific guidance to help PTSD patients effectively deal with traumatic memories. For example, more personalized and targeted treatment plans can be designed based on the active forgetting mechanism of PTSD patients. These programs not only focus on alleviating traumatic memories, but also include enhancing patients' cognitive memory abilities and emotion regulation strategies to improve overall treatment effectiveness and reduce the risk of relapse.

2. Characteristics and mechanism of active forgetting in patients with PTSD

According to previous research, active forgetting is divided into directed forgetting and suppression-induced forgetting [5]. Directed forgetting is the phenomenon where memories are impaired due to the disruption of the encoding process, whereas suppression-induced forgetting is the phenomenon where memories are impaired due to suppression during the retrieval phase [7]. Next, this study will elaborate on the characteristics and cognitive neural mechanisms of active forgetting in PTSD patients from these two types of active forgetting.

2.1. Characteristics of directed amnesia in PTSD patients.

The phenomenon of memory impairment caused by inhibition at the encoding stage is called directed forgetting [7]. The study of directed forgetting effects is inseparable from the directed forgetting paradigm. The directed forgetting paradigm mainly examines how people lead to forgetting by blocking and interfering with memory encoding [4]. It is divided into two stages - learning stage and testing stage [10]. According to the different positions where the cue words appear in the learning stage, the directed forgetting paradigm can be divided into item method and word list method. The item method is to present a memory or forgetting prompt word after the subject has read each learning item; while the word list method is to uniformly prompt the subject to remember or forget the previous items after the subject has read a series of items [9]. According to previous research, the generation of directed forgetting effect may be related to five aspects: (1) instructions promote the encoding of words that need to be remembered; (2) instructions inhibit the brain's encoding of words that need to be forgotten; (3) The subjects selectively rehearse the words they need to remember; (4) the subjects selectively rehearse the expected test words; (5) during the test, the subjects selectively search the memory of the words [5] . There are two explanations for the mechanism of directed forgetting. One is the selective encoding theory, that is, each item is in working memory before the cue word to remember or forget appears. After the cue word appears, the memory prompt will cause the subject to encode the item carefully, but the forgetting prompt will guide the subject to clear the item from working memory and not encode it carefully; the other is the inhibitory control theory, that is, a requirement to forget The cue causes subjects to generate positive, inhibitory-related brain activity that prevents further encoding of the item. Previous research reflects that when the project method is used, both theoretical mechanisms are involved in the process of directed forgetting [9].

The characteristics of directed amnesia in PTSD patients derived from previous studies are as follows: PTSD patients have a higher directed amnesia effect for emotional materials than neutral materials; patients with high-level PTSD have a higher directed amnesia effect than patients with low-level PTSD; patients with high-level PTSD have a higher directed amnesia effect than patients with low-level PTSD. Active recognition avoidance in the memory processing stage has a greater impact on the directed forgetting effect than the defect of retrieval inhibition [1]. The above study was conducted using the project method and used the TBR - TBF score as an indicator. Among them, the TBR score of emotional materials is higher than that of neutral materials, and the TBF score is lower than that of neutral materials. Therefore, in general, the TBR-TBF score of emotional materials is higher than that of neutral materials. The directed forgetting effect of PTSD patients on emotional materials is higher than that of neutral materials. The TBR-TBF scores of patients with high-level PTSD are higher than those of patients with low-level PTSD, and the TBF scores are lower than those of patients with low-level PTSD. PTSD patients. Therefore, in general, the TBR-TBF score of patients with high-level PTSD is higher than that of patients with low-level PTSD, and the directed amnesia effect is higher than that of patients with low-level PTSD. The TBF score of patients with high-level PTSD is significantly lower than that of patients with low-level PTSD. This difference in indicators can be used as a basis for high-level PTSD patients. There is evidence that active recognition avoidance in PTSD patients has a greater impact on the directed forgetting effect during the memory processing stage than the deficit in retrieval inhibition. The reason for reaching the above conclusion is that emotional materials pay more attention to the details of the stimulus during memory processing, negative materials tend to process specific items, and positive materials make subjects pay more attention to the commonality of the stimuli. In the competition for limited psychological resources during the learning process, negative stimuli can receive more attention and thus be processed with priority. When resources are sufficient, positive stimuli are also fully processed. Therefore, different emotional materials receive more attention than neutral materials in memory processing and retrieval; at the same time, high-level PTSD patients can more sensitively identify information that may cause mood swings to them than low-level PTSD patients, and actively block it. This information is used to protect yourself.

2.2. Characteristics of suppression-induced forgetting in PTSD patients

Suppression-induced forgetting is a memory impairment phenomenon caused by inhibition in the retrieval stage [7]. There are two types of suppression-induced forgetting paradigms - direct inhibition and vicarious inhibition. The commonly used paradigm of direct inhibition is the TNT paradigm, which is divided into learning phase, TNT phase and test phase. In the learning stage, subjects were asked to learn "cue-target" word pairs; in the TNT stage, only clue words were presented to subjects. The response condition (think) requires subjects to recall the corresponding target word, and the inhibition condition (no think) is required to avoid recalling the target words. Some of the learned words will not appear in this stage but will be compared with the experimental task words as the baseline level in the test stage; in the test stage, all words presented in the learning stage are tested. If the amount of information recalled under the NT condition is significantly lower than the baseline level, the suppression-induced forgetting effect occurs [12]. Vicarious suppression refers to limiting the brain's retrieval of irrelevant memories by increasing the cognitive resources occupied by dominant ideas. Start by learning cue-target word pairs. In the formal experimental stage, when the clue word is presented, the subject is given an alternative word, and the subject is required to remember the connection between the clue word and the alternative word, which helps the subject not to recall the target word [6]. Some studies have shown that people with high trauma experience have a stronger suppression-induced forgetting effect than those with low trauma experience. Because after experiencing trauma, individuals will continue to suppress the intrusion of traumatic memories, which may enhance the ability of memory suppression. In fact, damage to their memory suppression ability may cause PTSD patients to be unable to remove traumatic memories, and the repressive and amnesic effects are weakened or even eliminated.

There are two explanations for the theoretical mechanism of suppression-induced forgetting, one is the interference theory, and the other is the inhibition theory. Interference is said to be that under the

inhibition condition, the subject mixes the target word with no Think instructs to make connections, causing new connections to be made that compete with the previous memory content, thereby weakening it. There was an increase in memory-related activity; inhibition theory means that suppression is triggered as a direct response, and the subject selectively retrieves the target word. Hippocampal activity decreased during inhibition trials, and memory-related activity decreased. Some studies have shown that the suppression theory is correct, because under suppression conditions, subjects' memory-related activities are weakened rather than enhanced [14]. Some studies have also shown that in the TNT suppression and forgetting paradigm, the interference theory and the inhibition theory are compatible, because interference can capture clues and forget independently in the TNT paradigm. The difference in the hippocampus between the recall condition and the NT condition may reflect the enhanced activation under the recall condition, rather than the suppression of memory under the NT condition. Explanation of this relative difference depends on whether learning competition theory is used to process the data or inhibition theory [15].

2.3. *Neural mechanism of active forgetting*

According to previous research, the mechanism of suppression-induced forgetting in ordinary people is as follows: (1) As the intensity of repeated suppression of intrusions continues to decrease, the activation of the ventral attention network also decreases. At the same time, during the inhibition process, the functional connections between the executive control network increased significantly, the functional connections within the default network and visual cortex decreased, and the functional connections within the dorsal attention network increased [3]; (2) ALFF in a large number of frontal lobe areas related to executive control (Mainly including dorsolateral prefrontal lobe , supplementary motor area, superior frontal gyrus, etc.) and Reho (right dorsolateral prefrontal lobe, supplementary motor area, and orbitofrontal lobe) are positively correlated with active forgetting ability [6]; (3) dlPFC (dorsolateral prefrontal cortex area) and HC have opposite neural activity patterns under memory suppression conditions [6]; (4) Gray matter in the dorsolateral prefrontal area (DLPFC/BA9) and anterior cingulate location (ACC). There is an obvious negative correlation between volume and individual inhibition ability [4]; (5) The hippocampus, a memory-related brain area, and the amygdala, an emotional processing brain area, are inhibited [4]. This matches the reduced memory-related activity noted in the behavioural evidence, thus supporting the suppression theory as an explanation for the amnesic effect of suppression. The neural evidence of PTSD in previous studies mainly focused on suppression-induced forgetting, and there is a lack of neural evidence of directed forgetting. The neural mechanism of suppression-induced forgetting in PTSD patients is different from that of ordinary people. Patients with PTSD exhibit a different neural mechanism for suppression-induced forgetting compared to the general population. Their parietal-occipital-temporal network shows higher gamma energy under the 'no think' condition than under the 'think' condition. This implies that individuals with PTSD have an increased amount of recall under the 'no think' condition, and the effect of suppression-induced forgetting is diminished. [1]. Because the amount of recall is lower than the think condition and baseline level, the suppression-induced forgetting effect occurs. This is also consistent with the evidence mentioned above that the impairment of memory suppression ability may cause PTSD patients to be unable to remove traumatic memories. And the activity of the left middle frontal gyrus on the right side is lower than that of ordinary people [16], and the higher the WBSI score, the lower the activity of the left middle frontal gyrus on the right side. This shows that PTSD patients have higher WBSI scores than ordinary people. They suffer from uncontrollable intrusive thoughts and have weak suppression-induced forgetting effects. Additionally, increased amygdala activity explains the acquisition, maintenance, and expression of conditioned fear memories. The disinhibition of the amygdala by the medial prefrontal cortex is one of the reasons that promotes the emergence of PTSD. At the same time, the excitability of the frontal lobe is reduced. As mentioned earlier, the frontal lobe area is positively correlated with the ability to actively forget. That is, the stronger the neural activity in the frontal lobe, the stronger the ability to actively forget. Therefore, its ability of suppression-induced forgetting is weakened [13].

3. Discussion

The difference between directed forgetting and suppression-induced forgetting is that the former is a memory impairment phenomenon caused by inhibition in the memory encoding stage, and the latter is a memory impairment phenomenon caused by inhibition in the retrieval stage. Moreover, the directed amnesia effect of high-level PTSD patients is higher than that of low-level PTSD patients, while the suppression-induced forgetting effect of high-level PTSD patients is lower than that of low-level PTSD patients. This is because patients with high-level PTSD are more sensitive to emotional materials, and their strong self-protection motivation causes them to avoid memorizing emotional materials during the learning stage, distracting their attention, and selectively encoding emotional materials. Therefore, patients with high-level PTSD have a high forgetting effect in the encoding stage of memory, that is, a high directed forgetting effect. As for suppression-induced forgetting, patients with high-level PTSD have already memorized the material, and their poor inhibition ability makes them involuntarily recall the material. Therefore, patients with high-level PTSD have a weak forgetting effect in the memory retrieval stage, that is, a weak suppression-induced forgetting effect. The connection between directed forgetting and suppression-induced forgetting is that they both involve inhibition theory, that is, the subject generates inhibitory brain activity after receiving relevant instructions, thereby achieving the purpose of forgetting.

The shortcoming of existing research is that there is a lack of neural evidence for directed forgetting in PTSD patients, such as which brain areas' activities and connections are related to directed forgetting in PTSD patients, and how the activity and connections in these brain areas are different from ordinary people. At the same time, the sample size was small due to the limited number of people with high PTSD levels three and a half years after the earthquake. Only 70 samples that met the requirements were screened out. It would be more appropriate to increase the number to 100. And some studies did not exclude additional variables, such as subjects' depressive state scores. This may affect the subjects' feelings when seeing negative words and thus affect the active forgetting effect. And only neutral materials were used instead of emotional materials for research, such as studying the relationship between inhibitory ability and grey matter volume, which lacks generalizability. Because the addition of emotional materials will make the entire study more comprehensive and convincing. This is because it is not clear whether the conclusions obtained by using emotional materials are consistent with those obtained by using neutral materials.

4. Conclusion

This study summarizes active forgetting and its types and paradigms based on previous research. At the same time, it compares the neural mechanism of active forgetting in PTSD patients with ordinary people and summarizes the characteristics of active forgetting in PTSD patients: high degree of PTSD. The directed forgetting effect of patients was higher than that of patients with low-level PTSD, while the suppression-induced forgetting effect of patients with high-level PTSD was lower than that of patients with low-level PTSD. This is due to the different mechanisms of directed forgetting and suppression-induced forgetting in PTSD patients. Patients with high-level PTSD are more sensitive to emotional materials, and their strong self-protection motivation causes them to avoid memorizing emotional materials during the learning stage, distracting their attention, and selectively encoding emotional materials. And its poor inhibition ability makes it involuntarily recall the material. The significance of this study is that it can help better understand the pathogenesis of PTSD and develop effective treatment plans to help PTSD patients alleviate symptoms such as flashbacks, recurrences, and hypervigilance, so that they can better integrate into social life.

5. Prospects

According to the shortcomings of existing research, in the future, we can study the neural mechanism of directed forgetting in PTSD patients by exploring the activities and connections of brain areas related to directed forgetting in PTSD patients, to clarify their differences from ordinary people, and to understand their pathogenesis and treatment methods. . The sample size can also be increased in future

studies. A feasible method is to change the sample to a larger group. And excluding additional variables such as depression status scores makes the experimental results more accurate. At the same time, emotional materials are used to study the relationship between inhibition ability and gray matter volume, to clarify whether the conclusions of research using emotional materials and neutral materials are consistent, and to enhance the generalization of the research.

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Association between the BMI and depression symptoms in the U.S. older adults base on NHANES

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Abstract. Depression ranks among the most prevalent mental health conditions globally, with major depression being more common in elder people. Nonetheless, the link between depressive symptoms and body mass index (BMI) remains ambiguous. The purpose of this study was to investigate the correlation between BMI and symptoms of depression in American elders. In this paper, a cross-sectional study was conducted involving 7,769 participants aged 60 years or older, with all data collected from the 2005-2016 National Health Survey (NHANES). We employed a multivariate logistic regression analysis to evaluate the association between levels of BMI (as independent variables) and presence of symptoms of depression (as outcome variables). Depressive symptoms were primarily assessed using the Patient Health Questionnaire (PHQ-9). Additionally, in this paper, several sensitivity analyses are performed to verify the robustness of our findings. After adjusting for covariates, the final model shows the ORS(95%CI) of underweight and obese people are 1.52(0.78,2.98) and 1.34(0.94,1.93), respectively. And there is a non-linear and U-shaped relationship between the BMI and the depressive symptoms. Both underweight and obese people have a higher incidence of depression in elder people. To validate the association between depression and weight and their causal link, more research is required.

Keywords: Depression, Body Mass Index(BMI), Elder People, NHANES

1. Introduction

In more than 5% of people worldwide, depression is a common mental illness, according to the World Health Organization (WHO) [1]. Depression that strikes people 60 years of age or older is known as “late-life depression”. And it has been largely under-diagnosed and under-treated [2]. But it can have severe health outcomes, which include a greater risk of death, suicide, physical disability, and poor quality of life [3]. More than half of cases represent a first onset in later life [4]. From a public health perspective, since elderly depression tends to be chronic and/or recurrent, Insufficient identification and management of depression in elderly individuals lead to higher usage of health care services and impose a strain on both patients and their families.

Approximately one-third of the US population is obese, while another one-third is overweight [5]. Previous systematic reviews and meta-analyses have highlighted a bidirectional link between obesity and depression. It was shown that persons who were obesity exhibited a 55% elevated probability of encountering depression, whereas those who were depressed had a 58% heightened potential of developing obesity [6]. A potential explanation for this phenomenon could be attributed to several

prevalent risk factors associated with obesity and depression, including but not limited to low socioeconomic level, inadequate sleep, and the consumption of tobacco and alcohol, which may contribute to the prevalence of both diseases [7].

While there is a well-established connection with obesity, the association between Body Mass Index (BMI) and depression does not display consistent patterns. Research presents varied findings: certain studies suggest a direct positive correlation with higher BMI elevating depression risk, yet this link appears confined to individuals with a BMI in the range of severe obesity (35-39.9 or higher) in other studies [8]. Moreover, a subset of the research suggests a non-linear, U-shaped correlation between BMI and sign of depression, particularly noted in adults over the age of 50 up to centenarians [9].

The primary objective of this study was to investigate the possible association between body mass index (BMI) and depression among individuals aged 60 years and above in the United States, while considering the potential influence of different confounding factors, in light of the diverse results reported in other studies.

2. Methods

2.1. Survey Description

The National Center for Health Statistics, a division of the Centers for Disease Control and Prevention, is responsible for administering the National Health and Nutrition Examination Survey (NHANES), a population-based, cross-sectional survey aimed at estimating the health and nutritional status of the American people. The poll used a sophisticated multi-stage stratified methodology that oversampled older individuals and members of underrepresented groups. Highly skilled personnel conducted the examinations and interviews, and automated data gathering methods were applied. The respondent and, if required, the respondent's proxy were the targets of questions. To ensure a sufficient sample size for our analysis, we utilized data from the National Health and Nutrition Examination Survey (NHANES) collected during the cycles of 2007–2008, 2009–2010, 2011–2012, 2013–2014, and 2015–2016. The NHANES study protocol received approval from the Research Ethics Review Board of the National Center for Health Statistics, with all participants providing signed informed consent. For more comprehensive information on the NHANES, including questionnaires, datasets, and related documentation, please visit the NHANES website at the Centers for Disease Control and Prevention ([cdc.gov](https://www.cdc.gov)) [10].

2.2. Study population

From 5 cycles, of the 50,588 participants interviewed by NHANES between 2007 and 2016, 9,760 participants over the age of 18 qualified as a subsample for this study. And after excluding individuals with an unfilled depression score and other covariates, the final number of participants is 7831. See the flowchart below figure 1.

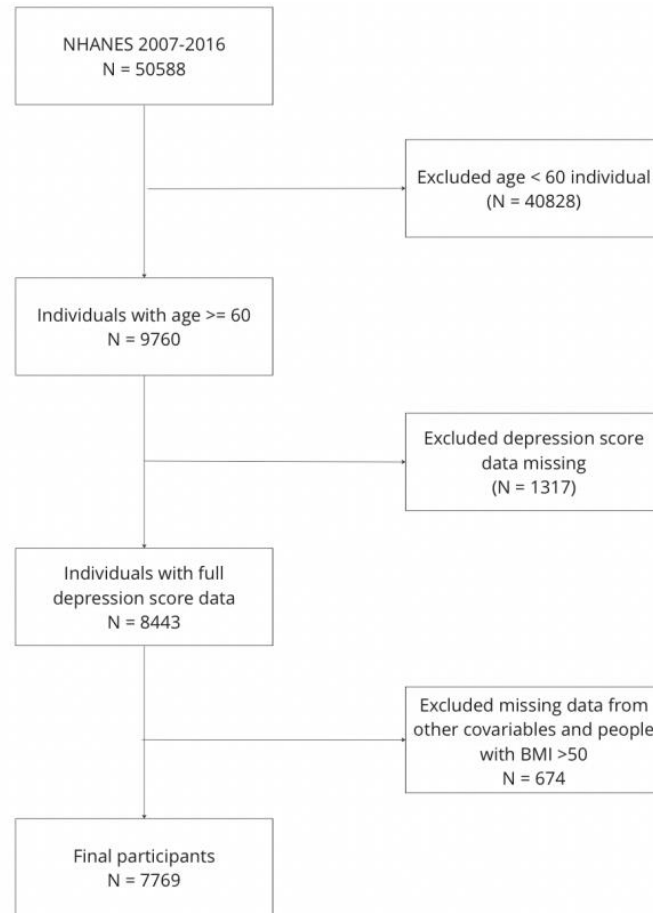


Figure 1. Flowchart of participants inclusion (National Health and Nutrition Examination Survey [NHANES])

2.3. Measurement

2.3.1. Outcome variables. The Patient Health Questionnaire (PHQ-9), which is used to assess depression within the past two weeks, is a brief but effective tool for assessing depressive symptoms. The questionnaire consists of nine items that reflect the signs and symptoms listed in the Diagnostic and Statistical Manual of Mental Disorders, with each item scored on a scale from 0 to 3 (0 = none, 1 = a few days, 2 = more than half the time, and 3 = almost every day), with a total score ranging from 0 to 27. According to previous studies, a PHQ-9 score of 10 or more usually indicates depression. The sensitivity and specificity for diagnosing major depression is 88% [12]. In the present study, depressive symptoms were coded as 0 (no depression, 0-9 points) or 1 (depression, 10-27 points) based on this threshold.

2.3.2. Exposure measurement. By dividing weight in kilograms by height in meters squared and rounding to the nearest decimal point, one can compute body mass index (BMI). The WHO recommendations [13] served as the basis for the cut-off criteria that were employed. Subjects were classified as underweight if their body mass index (BMI) was less than 18.50 kg/m², normal if it was between 18.50 and 24.99 kg/m², overweight if it was between 25 and 29.99 kg/m², and obese if it was greater than 30 kg/m².

2.3.3. Covariate assessment. The prior literature served as the basis for the covariate selection. Data on age, gender, race/ethnicity, education, marital status, annual family income, recent tobacco use, recent alcohol issues, length of sleep, hypertension, and diabetes were gathered using standardized questionnaires. An ongoing variable was age, expressed in years. Either male or female was listed as the gender. There were four categories for race/ethnicity: non-Hispanic black, non-Hispanic white, and other. Education was classified into three levels: not completing high school, completing high school or an equivalent, and completing higher education. The two categories of marital status were single and cohabiting. The three categories of annual household income were <\$25,000, \$25,000–\$75,000, and ≥\$75,000.

Recent smoking status was established by responses to: “Do you now smoke cigarettes?” and “Have you smoked at least 100 cigarettes in life?” Respondents who indicated “some days” or “every day” to the first question and “yes” to the subsequent question were categorized as active smokers. Regarding recent drinking problems, participants were requested to ask “On how many days in the past 12 months have you had 4 (female)/5 (male) drinks or more of alcoholic beverages? Participants who answered ≥1 were judged to have had a recent drinking problem.

Weekday or weekday sleep duration was self-reported by participants. For the 2007-2014 cycle, sleep duration was calculated from a question posed to NHANES participants about the participant’s daily sleep duration: “How much sleep do you get (in hours)?” In the 2015-2016 cycle, sleep duration was determined based on the response to the query, “How much sleep do you typically get on weekdays or nights during the week?” The categories for sleep duration were less than 7 hours, 7 to 9 hours, and 9 hours or more. The presence of diabetes and hypertension was ascertained by inquiring with participants whether a doctor or healthcare professional had ever diagnosed them with diabetes or hypertension.

2.4. Statistical analysis

Weights, clusters, and strata were adjusted using the survey settings. Frequencies and percentages were computed for categorical variables; all estimates and 95% confidence intervals (95%CI) were weighted to ensure that they were nationally representative. The analysis incorporated the Mobile Examination Center (MEC) sample weights and the relevant house investigated sample design factors (strata, primary sampling unit) to accommodate the intricate survey design. Table 1 illustrates the major demographic and clinical characteristics of the participants involved in the study.

Table 1. Baseline Characteristics of Cohort

Variable	Depression	Normal	P-value
Age, in years	68.36(0.36)	69.68(0.14)	< 0.001
Gender:			< 0.0001
Female	374(7.57)	3559(92.43)	
Male	229(4.73)	3607(95.27)	
Race/Ethnicity:			< 0.0001
Hispanic	218(12.62)	1514(87.38)	
Non-Hispanic Black	112(7.41)	1471(92.59)	
Non-Hispanic White	244(5.58)	3669(94.42)	
Other	29(6.40)	512(93.60)	
Education:			< 0.0001
≤High school	287(11.59)	2042(88.41)	
>High school	193(4.47)	3433(95.53)	
High school	123(6.53)	1691(93.47)	
Marital Status:			< 0.0001

Table 1. (continued)

Living alone	343(9.08)	2897(90.92)	
Living together	260(4.72)	4269(95.28)	
AHI(Annual Household Income)			< 0.0001
<25000	347(12.10)	2457(87.90)	
>75000	33(2.03)	1435(97.97)	
25000-75000	223(5.67)	3274(94.33)	
Recent Smoking:			0.002
non-smoker	249(5.00)	3540(95.00)	
Recent Smoking	354(7.50)	3626(92.50)	
BMI Category:			< 0.0001
Underweight	11(8.34)	91(91.66)	
Normal	113(4.86)	1730(95.14)	
Overweight	179(4.88)	2651(95.12)	
Obese	300(8.45)	2694(91.55)	
Hypertension:			0.002
Hypertension	422(7.23)	4373(92.77)	
Normal	181(4.93)	2793(95.07)	
Diabetes:			< 0.0001
Diabetes	222(10.34)	1618(89.66)	
Normal/Borderline	381(5.29)	5548(94.71)	
sleep:			0.01
<7h	384(7.13)	3927(92.87)	
7h-9h	169(4.93)	2825(95.07)	
>9h	50(8.59)	414(91.41)	
Drinking Problem Recently			< 0.0001
Have drinking problem recently	66(4.82)	747(95.18)	
Normal	192(4.70)	3166(95.30)	
Unknown	345(8.75)	3253(91.25)	

Note. Means $\pm$ standard errors (SE) are presented for continuous variables, counts (weighted percentages) for categorical variables.

The investigation used logistic regression analysis to examine the link between depression symptoms and Body Mass Index (BMI) through three separate models related to BMI figures. The first model, Model 1, did not include adjustments for any covariates. Model 2 was modified to reflect adjustments for age, gender, and race. Model 3, on the other hand, included adjustments for all conceivable covariates.

The statistical software packages R were utilized to conduct all of the analysis. (<http://www.R-project.org>, The R Foundation) and Free Statistics software versions 4.3.1.

3. Results

Our comprehensive analysis revealed significant associations between various demographic and behavioral factors with depression status among the studied population, as shown in Table 1. Our dataset comprised 3933 females and 3836 males aged 60 years and older. The prevalence of depression varied significantly across gender, with females and males affected at rates of 7.57% and

4.73%, respectively. The mean age for participants without depression was 69.68 years (SE±0.14), whereas those with depression were slightly younger, with a mean age of 68.36 years (SE±0.36).

For race/ethnicity, individuals identifying as Hispanic (12.62%) and Non-Hispanic Black (7.41%) exhibited higher rates of depression compared to Non-Hispanic White (5.58%) and other ethnicities (6.40%), indicating notable disparities ($p < 0.0001$). Educational attainment also showed a strong correlation with depression; participants with less than a high school education had a significantly higher incidence of depression (11.59%) compared to those with at least a high school diploma (4.47%) or high school graduates (6.53%).

Marital status was another significant factor; those living alone had a higher prevalence of depression (9.08%) in contrast to those living with others (4.72%), indicating the potential impact of social support on mental health ($p < 0.0001$). Analysis of AHI revealed that individuals with an AHI greater than 75000 had a depression rate of 2.03%, which was lower compared to those with AHI less than 25000 (12.10%) with a p-value of $p < 0.001$.

Recent smoking behaviors were also associated with depression. Current smokers comprised 7.50% of depressed individuals, which was significantly higher than non-smokers (5.00%, $p = 0.002$). Body Mass Index (BMI) categories showed a strong association with depression, with the highest prevalence found in obese individuals (8.45%, $p < 0.0001$).

Furthermore, hypertension and diabetes were more prevalent in the non-depressed group (4.93% and 5.29%, respectively) compared to the depressed group (7.23% and 10.34%, respectively), which may point to the complex interplay between these chronic conditions and mental health (hypertension $p = 0.002$, diabetes $p < 0.0001$).

Sleep duration was also correlated with depression, with those reporting less than 7 hours of sleep showing a higher prevalence of depression (7.13%, $p = 0.01$). Lastly, recent problems with drinking were more prevalent among the depressed (4.82%) compared to those without (0.70%), indicating the significant relationship between alcohol use and depression ($p < 0.0001$).

This table shows the multifaceted nature of depression and highlights the importance of considering a wide range of demographic and lifestyle factors when addressing mental health concerns in older adults.

Table 2. BMI and Depressive Symptoms in Female and Male Subgroups by age categories

	overall	60-69 years	70-79 years	80+ years
BMI:				
Females	29.00±0.16	29.55±0.22	29.35±0.23	26.80±0.21
Males	28.93±0.13	29.34±0.19	28.92±0.20	27.29±0.19
Depression Symptoms:				
Females	374(7.57)	237(9.16)	93(6.13)	44(5.43)
Males	229(4.73)	139(5.50)	64(3.69)	26(3.82)

Note. Means ± standard errors (SE) are presented for continuous variables, counts (weighted percentages) for categorical variables.

In Table 2, the data highlights gender disparities in both Body Mass Index (BMI) and the prevalence of depression symptoms across different age groups. The overall mean BMI was 29.00±0.16 for females and 28.93±0.13 for males, suggesting a marginal difference with females having a slightly higher mean BMI. When examined across age groups, there was a trend of decreasing BMI with increasing age: females aged 60-69 years had a mean BMI of 29.55±0.22, which steadily declined to 26.80±0.21 in those 80 years and older. A similar pattern was observed in males, with those aged 60-69 years having a mean BMI of 29.34±0.19, decreasing to 27.29±0.19 in the 80+ cohort.

Depression symptoms followed a different trend, with a higher prevalence in females (7.57%) compared to males (4.73%) in the overall cohort. Within the age categories, females aged 60-69 years

had the highest prevalence of depression symptoms (9.16%), which decreased to 5.43% in those 80 years and older. Males aged 60-69 years had a depression prevalence of 5.50%, which also decreased with age to 3.82% in those 80 and older. The data shows a trend of declining depression symptoms as age increases for both sexes, with the decline being more noticeable in women.

Table 3. Association between BMI and depression symptoms

	Model 1 OR(95% CI)	p-value	Model 2 OR(95% CI)	p-value	Model3 OR(95% CI)	p-value
BMI	1.05(1.03,1.07)	<0.0001	1.04(1.02,1.06)	<0.0001	1.02(1.00,1.05)	0.03
BMI Category						
Underweight	1.78(0.88,3.60)	0.11	1.79(0.87,3.67)	0.11	1.52(0.78,2.98)	0.22
Normal	1.0	ref	1.0	ref	1.0	ref
Overweight	1.00(0.71,1.42)	0.98	1.02(0.72,1.45)	0.90	0.96(0.67,1.36)	0.80
Obese	1.81(1.26,2.60)	0.002	1.71(1.18,2.50)	0.01	1.34(0.94,1.93)	0.11

Model 1: No covariates were adjusted.

Model 2: adjust for: gender; age (smooth); race/ethnicity

Model 3: adjust for: year; gender; age (smooth); race/ethnicity; marital status; AHI; hypertension; diabetes; recent smoking; education levels; recent drinking problem; sleep duration

Table 3 demonstrates how three progressively modified models were used to examine the relationship between body mass index (BMI) and the signs of depression. An odds ratio (OR) of 1.05 (95% Confidence Interval [CI]: 1.03, 1.07; $p < 0.0001$) for Model 1, which did not account for any variables, demonstrated a substantial correlation and showed that there was a 5% increase in the likelihood of depression symptoms for every unit rise in BMI.

Upon adjustment for gender, age, and race/ethnicity in Model 2, the association remained significant, albeit slightly attenuated, with an OR of 1.04 (95% CI: 1.02, 1.06; $p < 0.0001$). This suggests that demographic factors do mediate the relationship between BMI and depression, but a significant independent effect of BMI persists.

In Model 3, which included a comprehensive set of covariates—year, gender, age (smooth), race/ethnicity, marital status, AHI, hypertension, diabetes, recent smoking, education levels, recent drinking problem, and sleep duration—the association was further attenuated yet remained significant (OR = 1.02; 95% CI: 1.00, 1.05; $p = 0.03$). This indicates that while the effect size of BMI on depression is reduced when controlling for these variables, there is still a discernible impact of BMI on depression outcomes.

If we treat BMI as a category variable, the three models give different results. In Model 1, which does not adjust for any covariates, both underweight and obese categories show increased odds of depression symptoms compared to the normal BMI category, with obese individuals having significantly higher odds (OR 1.81, 95% CI [1.26,2.60], $p=0.002$). In Model 2, the association between obesity and depression remains significant (OR 1.71, 95% CI [1.18,2.50], $p=0.01$), while the association for the underweight category is not significant ($p=0.11$). In contrast, the odds for obese individuals are still higher but the association is no longer significant (OR 1.34, 95% CI [0.94,1.93], $p=0.11$) in Model 3. This indicates that the covariates added in Model 3 can explain some of the relationships between BMI and depression.

The generalized additive model (GAM) shown in Figure 2 illustrates the U-shaped relationship between body mass index (BMI) and sign of depressive. The analysis accounts for a series of covariates, including gender, age (adjusted using a smoothing function), race/ethnicity, marital status,

AHI, hypertension, diabetes, recent smoking habits, education levels, recent drinking problems, and sleep duration. The red line in the graph denotes the smoothed mean estimate of the probability of depressive symptoms across the BMI spectrum, while the shaded area indicates the 95% confidence interval. The U-shaped curve suggests that both lower and higher values of BMI are linked to an increased likelihood of experiencing symptoms of depression, with the nadir occurring in the intermediate BMI range. This pattern persists even after adjusting for the listed confounding variables, suggesting a complex and potentially bidirectional relationship between body weight and mental health.

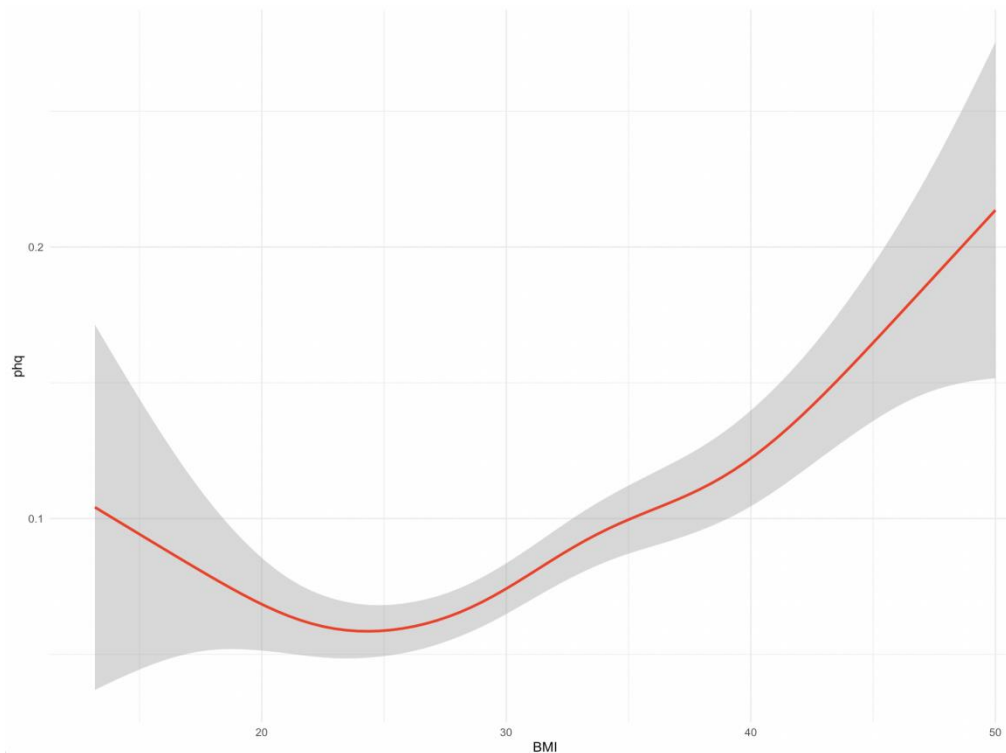


Figure 2. GAM

The relationship between depressive symptoms and BMI, after adjusting year; gender; age (smooth); race/ethnicity; marital status; AHI; hypertension; diabetes; recent smoking; education levels; recent drinking problem; sleep duration.

4. Discussion

In order to investigate the association between Body Mass Index (BMI) and the prevalence of depressive symptoms among American adults, we conducted a community-based cross-sectional investigation for this study. Utilizing a strong methodological framework, we gathered and examined data from a wide range of participants in various communities. According to our research, there is a U-shaped curve in the relationship between BMI and depressed symptoms, meaning that there is a higher risk of depression associated with both lower and higher BMI extremes. The constant observation of this tendency across multiple demographic groups suggests that it is a universal trend, independent of factors such as age, gender, or financial situation. Notably, our findings support a previous study's conclusion that there is a complex and nonlinear association between them. Similar to the previous study, this one also found a U-shaped correlation, indicating that being underweight and being obese may be risk factors for depression. By offering additional empirical data from a wider and more diverse American population, our study expands on these findings and emphasizes the

significance of taking BMI into account when conducting mental health examinations and interventions.[13].

Our results corroborate the significant, independent relationship between depression and BMI. This study provides more evidence that numerous mental health issues and physical conditions in older persons are connected due to their biopsychosocial character. Our research highlights the potential usefulness of depression and BMI measurements in evaluating changes in the mental and physical well-being of older individuals who live in the community.

Previous studies shown that the relationship between depression and obesity is bidirectional. While research from Western nations typically indicates a positive correlation between obesity and depression, studies from Asian regions like China and Korea have demonstrated a negative correlation amongst the elderly population [14-15]. A potential reason for the disparate findings could be that factors like gender, age, socio-economic background, ethnic identity, citizenship and particularly culture, influence the connection between obesity and depressive disorders. Last but not least, other psychiatric disorders should also be explored in future research, as previous studies have revealed that not only depression is associated with obesity, but other psychiatric disorders are also associated with obesity [16].

In the study, we can see that the obese people and the lean people both have a higher odds ratio than the normal people. This may be caused by social attributes, as they are more likely to experience weight discrimination [17]. This implies that society ought to assist obese individuals more and normalize the attitude of the general public toward obesity. In addition, poor eating patterns (including those that follow sadness), behavioral disorders, and adverse medical or socioeconomic circumstances are linked to depression.

This research is subject to certain constraints. Initially, the data collection relied on self-administered questionnaires and subjective self-reporting, which could introduce errors stemming from misremembering or biased reporting. Additionally, the efficacy of BMI as an indicator of body fat has been a topic of debate. While BMI is commonly used to evaluate weight in the general populace, it may not accurately reflect levels of visceral fat, which can be either overestimated or underestimated.

5. Conclusion

In summary, there is a significant correlation between both underweight and overweight conditions and depression, exhibiting a U-curve association with Body Mass Index (BMI). Further research is warranted to investigate the connection between body weight aspects, such as visceral fat and its distribution, and depression. As this research is a cross-sectional study, we can not explore the causal link between BMI and depression. This needs further study. Also, the relationship can be temporally, as the data are not collected longitudinally. For the results of the 3 model, some of the odds ratios have a really high p-value, which indicates that there is some interaction or correlation between the selected covariates, which needs more consideration. Furthermore, the sample may not represent the whole population well, as selecting participants may have bias. And not all the potential confounders have been included in the analysis.

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Evaluation of traditional lung cancer detection modalities and research and application of biomarkers in the early detection of lung cancer

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Abstract. At present, the cancer that kills people the fastest worldwide is lung cancer, and many lung cancer patients are found or detected at the late stage of lung cancer, which is very bad for the prognosis of the disease, and makes the mortality rate of the patients increase greatly. Therefore, the development of techniques for the early diagnosis and detection of lung cancer is imperative. At the moment, lung cancer can be detected early due to biomarkers. This review primarily outlines the many kinds of biomarkers and the conventional techniques for detecting and diagnosing lung cancer. In addition, bodily fluids' potential application as biomarker carriers in the detecting of cancer development and progression is mentioned in this review, the feasibility of non-invasive cancer diagnostic methods is analyzed, and the current status of the development of such diagnostic methods is summarized.

Keywords: lung cancer, early detection, body fluid, non-invasive diagnosis, biomarkers

1. Introduction

Lung cancer, one of the world's most common and fatal illnesses, is often diagnosed in advanced stages [1]. According to Goldberg et al., early diagnosis of all lung cancers in the United States could reduce annual deaths by more than 70,000 per year [1]. According to Reem Nooreldeen and Horacio Bach, lung cancer will be the leading cause of cancer worldwide by 2050, accounting for half of all cases [2]. Therefore, early detection of lung cancer is urgent, as it allows for treatment with today's medical methods. Many studies have shown that lung cancer is usually symptomless in its early stages, and most patients are diagnosed with this disease at an advanced stage. As a result, the progression of the disease is often more advanced than it should be, leading to a poor prognosis, having a survival rate of below 20% following five years [3]. Accurate and effective early detection methods are needed. Nowadays, some detection methods have been proposed and applied to the early identification of lung cancer, while these techniques are never without limits, which lead to the error of the test results, affecting the accuracy of the test results. The current detection methods, whether traditional or early detection methods, make it difficult to avoid the immersion test, which has a certain impact on the patient's lesion and requires the assessment of the patient's indicators and his/her ability to bear before sampling the biopsy specimen for detection, which is a high-risk and costly detection method. Non-invasive detection methods are under development.

Numerous investigations have revealed that the application of lung cancer biomarkers as indicators of detection is a promising method. Therefore, a summary of the traditional detection methods, the methods of early lung cancer detection proposed by the current research frontiers, and an analysis of the advantages and disadvantages of each detection method will be conducive to the discovery of the advantages of the detection in different ways, and will serve as a basis and reference for future research in related fields.

2. Lung Cancer Incidence and Mortality

Lung cancer has among of the highest incidence and fatality rates in the globe in recent years. Jacques Ferlay et al. reviewed the sources of data and techniques employed in the 2020 GLOBOCAN cancer statistics published by the International Agency for Research on Cancer (IARC). The data showed that with non-melanoma skin cancer excluded, there were an estimated 19.3 million (95% uncertainly interval [UI]: 19.00-19.6 million) new cases of cancer globally in 2020. The cancer death rate was almost 10.0 million (95% [UI]: 9.7-10.2 million, 9.9 million excluding non-melanoma skin cancer). One of the most important finding was that the majority of cancer-related deaths (1.79 million deaths) were caused by lung cancer [4].

Rebecca L. Siegel et al. found that higher cancer deaths than breast, prostate, and pancreatic cancers combined, and 2.5 times higher than colorectal cancer (CRC), are expected to occur in America in 2022—1,918,303 new cases and 609,360 cancer deaths, including around 350 deaths every day. Thus, the primary cause of cancer-related mortality remained lung cancer. There were researches which illustrated state-by-state incidence rates of particular malignancies in the US from 2014 to 2018 and the morality rates for selected cancers in the US from 2015 to 2019 separately. The tables also reflect that both the cases and deaths of lung cancer were the most common [5].

Rebecca L. Siegel et al. said in 2023, the data for it was anticipated that American cancer mortality and new cases would be about 1,958,310 and 609,820 respectively. There were a number of data points that estimated incidence of selected cancer by states in the US in 2023 (approximately 238,340 cases) and the deaths for selected cancers in the US in 2023 (about 127,070 cases) separately [6].

Some data also suggests that the total rate of lung cancer has a downward trend from year to year because of the lung cancer early diagnosis. Additionally beneficial to the prognosis and prevention of lung cancer is early detection of the disease mortality. Rebecca L. Siegel et al. put forward the idea that earlier diagnosis has a significant influence on the result of lung cancer. The survival rate of patients increases, with the 5-year relative survival rising to 33% for regional stage and 60% for localized stage diseases, from 6% for distant-stage disease [5]. Many factors, such as , smoking, age, genes and so on, can increase the risk of suffering from lung cancer. As such, Matthew B. Schabath and Michele L. Cote (2019) supposed that the only option for those who were at a high risk to improve survival was early detection [7]. Reem Nooreldeen and Horacio Bach (2021) said that the reasons why the most common cause of cancer-related deaths was lung cancer which were not to be found until the cancer had progressed to a severe stage [2].

3. Advantages and Disadvantages of Contemporary Approaches to Lung Cancer Diagnosis and Mechanism

3.1. Radiographic Screening

Reem Nooreldeen and Horacio Bach (2021) summarized that the results of some researches showed that with the help of low-dose helical computed tomography (LDCT), the incidence of lung cancer decrease 20% in lung cancer as compared with the standard radiography screening. At the same time, the when the National Lung Screening Trial (NLST) compared yearly low-dose computed tomography (LDCT) screening versus traditional chest radiography, it was discovered that, after just three screening rounds, LDCT screening reduced the risk of lung cancer by 20% [8]. One of the advantages of LDCT is that compared with plain or conventional radiography screening, which is used to screen the whole lung, CT is more effective in screening the surrounding lesion around the lung. Spiral CT scanning can

continuously acquire data, so the screening time will be shorter with lower radiation exposure and the diagnosis accuracy of this technique is better than that of plain radiography. What's more, in just one or two breath holds, this technique can picture the entire chest, and this function can reduce artifacts that yield a more precise result in missing nodules that measure between 1 and 5 mm. Strong biomarkers would ideally enhance image-based screening in two ways [8]. The challenges of this technique are high cost and accessibility [2]. Reem Nooreldeen and Horacio Bach (2021) said that about 40% of all individuals enrolled in the National Lung Screening Trial's LDCT experienced at least one false positive result with a false-positive rate as high as 96%. The high rate of false positives can lead to high-cost screening and invasive diagnosis for patients. LDCT exists with the possibility of a false positive, so patients have to undergo an invasive check, which will increase the risk of surgery and complications. In other words, this technique cannot avoid the invasive check [2].

3.2. Sputum Examination

3.2.1. The Cytological Examination of Sputa. Although Cytological Examination of Sputa can aid in the identification of core tumors from the bigger bronchi, including small- and squamous-cell carcinomas, there are still some limitations to this technique. Small adenocarcinomas with a diameter of less than or equal to 2 cm can aid in the detection of central tumors from the bigger bronchi, including small-cell and squamous cell carcinomas, which are not visible in sputum samples, and these small adenocarcinomas originated from the airway ramifications. Based on the research of screening, the sensitivity of Cytological Examination of Sputa to early lung cancer is only 20% to 30%. Thus, the accuracy and sensitivity of this technique cannot be considered into the common detection of lung cancer [2].

3.2.2. Immunostaining. Immunostaining can deliver a better result than sputum cytology. Reem Nooreldeen and Horacio Bach (2021) mentioned that this detect method can predict two years before a clinical diagnosis of lung cancer X-ray and cytology-based. Other research found one of these antibodies (703D4), which was subsequently found to be capable of identifying the heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 protein with higher sensitivity. And the study's findings demonstrated that compared with routine X-ray of the chest and sputum cytology, the sensitivity of the early identification of lung cancer, hnRNP A2/B1 was 2- to 3- fold [2].

3.3. Bronchoscopy

White light bronchoscopy (WLB) is the most common technique used to diagnose lung cancer. However, this method exists a significant limitation on diagnosing pre-malignant lesions, and an expert bronchoscopist could only identify 29% of these cases. While fluorescence bronchoscopy may be able to help with this issue, there were some challenges in the detection of dysplasia. Thus, based on the mechanism that showed that dysplastic and malignant tissues exhibit lower autofluorescence signals than normal tissues, that is why the LIFE-lung Fluorescence Endoscopy was created. Although LIFE bronchoscopy has a high diagnostic sensitivity in detecting premalignant and early malignant lesions, the results are less specific, in other words there are more false positive results [2].

3.4. Lung Tissue Biopsies

Tissue biopsies are considered the gold standard for cancer diagnosis. Fiber optic bronchoscopy with or without transbronchial needle aspiration, endobronchial ultrasonography, image-guided trans-thoracic needle aspiration, mediastinoscopy, pleural fluid analysis (thoracentesis), thoracoscopy, and surgical techniques are among the many frequently used procedures in the diagnosis of lung cancer. However, the disadvantages of these methods were high cost, susceptibility to complications, and the need for larger samples [1]. Plasma testing has many advantages over biopsy as a test for common lung cancer NSCLC, such as being noninvasive, fast, and easily repeatable over time, but they can't be used as stand-alone testing for NSCLC patients since they can be less sensitive than tissue-based assays [9].

4. Types of Biomarkers of Cancer

4.1. Genetics and Epigenetic Biomarkers

4.1.1. P53. The P53 gene codes for a tumor suppression protein that is involved in various cell processes, such as cell cycle regulation, DNA repair, and apoptosis. Numerous P53 point mutations were discovered and assessed as potential indicators of NSCLC prognosis [3].

4.1.2. Epidermal growth factor reporters (EGFR). Some studies showed that about 62% of NSCLC cases had mutant versions of EGFR present or overexpressed. Furthermore, EGFR mutations have been linked to between 50% and 70% of lung cancer incidences in East Asia, suggesting that lung cancer biomarkers exist [3].

4.1.3. Ras Genes. The Ras family, which includes K-ras, H-ras, and N-ras, is in charge of producing proteins that are essential for signal transduction and cell division. Additional studies showed a high correlation between 78% of lung cancer patients and K-ras mutations. Furthermore, it has been demonstrated that circulating K-ras mutant proteins are significant prognostic and predictive indicators for lung malignancies [3].

4.1.4. Micro RNAs. MicroRNAs are RNA molecules containing 19–25 nucleotides that play significant roles in the silencing of genes. These tiny chemicals work by obstructing or damaging mRNA molecules, which ultimately suppresses the expression of those molecules. It has been demonstrated that these molecules have important roles in both the prognosis of cancer and cell development. They were also found to be relevant to various cancers' pathological parameters and this discovery showed that these molecules had the potential to be cancer biomarkers. MicroRNAs' existence in bodily fluids suggests they have the potential to be biomarkers for cancer diagnosis. These molecules may have opportunities for research breakthroughs in non-invasive detection of lung cancer [3].

4.2. Protein Based Biomarkers

While there are several established protein biomarkers for lung cancer, the presence of these biomarkers, and more specifically their expression level, varies significantly among different subtypes of the disease. The combination of some biomarkers succeeds in diagnosing excellent sensitivity and specificity in cases of lung cancer [3].

4.2.1. Carcinoembryonic Antigen (CEA). Several studies have shown a strong association between NSCLC survival and CEA values.

The research found a strong association between NSCLC and CEA levels. According to additional research, the prognosis following surgery is worse the higher the serum CEA level. The higher the value, the poorer the prognosis after surgery. Nowadays, CEA levels are taken into account in addition to other markers including CA 15-3, CCAT2, and AFP for a more accurate forecast [3].

4.2.2. Neuron-specific Enolase (NSE). The other prevalent lung cancer biomarker, neuron-specific enolase (NSE), was discovered to be elevated in the serum of patients with small cell lung cancer (SCLC). At this point, NSE is regarded as one of the most accurate biomarkers with a high diagnostic yield in SCLC cases. While monitoring the NSE as a marker in SCLC is enough for diagnosis, it is preferable to take into account additional biomarkers, such as CEA and CYFRA 21-1, in addition to this enzyme in order to achieve reliable detection in NSCLC [3].

4.2.3. Annexin A2 (ANXA2). The 36 kDa protein known as annexin A2 has been found to be a new and effective biomarker for lung cancer. It gained recognition as a lung cancer biomarker due to its notable rise in the disease's early stages. Current research found that, compared with healthy people, patients' levels of ANXA2 in serum were higher. In another experiment, heat shock protein 60 (HSP60) and

ANXA2 as a novel combination of lung cancer biomarkers showed significant improvements in lung cancer early diagnosis. All of the above illustrates that ANXA2 is promising in early lung cancer detection [3].

4.2.4. Serum Amyloid A1 (SAA1). This protein is regarded as one of the acute phase proteins, exhibiting high levels of expression in response to various severe conditions, infections, and traumas. Some experiments have shown that lung cancer patients have higher levels of SAA1, and that SAA1 levels in samples from cancer patients are fourteen times higher than in healthy people compared to normal people. It demonstrated a notable advancement in the early identification and diagnosis of lung cancer [3].

4.2.5. Breath and Volatile Organic Compound (VOC) Analysis. Every intracellular pathophysiological fluctuation has an impact on cellular metabolism and, consequently, on the products that are expelled, such as volatile organic compounds (VOCs). Breath volatile organic compounds (VOCs) may be an indicator of the body's biochemical state for lung cancer as well as lung disorders for which the patient has already received evaluation, such as asthma or chronic obstructive pulmonary disease. Phillips and colleagues, almost 20 years ago, found a possible correlation between breath composition and lung cancer with the analysis of 108 individuals. Furthermore, Phillips, using a model of 30 volatile organic compounds (VOCs), lung cancer was detected with a sensitivity of 84.5% and a specificity of 81.0% in a research involving breath samples from 193 lung cancer patients and 211 healthy controls. GC-MS has shown to be a helpful analytical method for identifying particular biomarkers in breath, but because of its complexity, long processing time, requirement for trained operators, and expensive cost, its potential application as an early diagnostic tool is limited. Furthermore, a number of studies show that it is extremely challenging to pinpoint a particular molecule that is associated with lung cancer. Rather, the ability to detect cancer depends on the relative amounts of many substances. These factors led the researchers to concentrate their efforts on novel techniques that were more easily accessible and economically viable [7].

4.2.6. Tumor Exosomes. Exosomes are a type of nanoscale extracellular vesicles (30–150 nm) that can infiltrate blood, plasma, and urine, among other bodily fluids. They are released from their parent cells. As a novel class of cancer biomarker, tumor exosomes have emerged as a viable marker for non-invasive early cancer diagnosis. Growing evidence has shown that tumor exosomes play important roles in the spread and progression of cancer by delivering abundant malignant bioinformation, including particular RNAs and proteins for facilitating intercellular communication. However, because of the characterization of exosomes, it is currently very difficult to identify tumor exosomes with good sensitivity and selectivity due to their small size and low concentration in bodily fluids. Therefore, it is imperative to devise dependable and efficient methodologies for the direct extraction and examination of exosomes related to cancer in intricate biological specimens, particularly in authentic hematic samples. This endeavor is anticipated to yield novel pathways for non-invasive, preemptive cancer diagnostics.

The limitations of currently investigated methods for exosome determination, most of which are limited by cumbersome sample handling techniques, and the difficulty of these methods to selectively detect tumor exosomes, have seriously hampered their widespread application in complex biological systems. There are also newer strategies that have excellent performance, but most of them require the use of ultracentrifugation to enrich exosomes. Technologies based on aptamers, cholesterol, and antibodies have now been cleverly suggested for the quantitative analysis and collection of tumor exosomes. Specifically, they can recognize and bind the biomarkers on the surface of malignant exosomes. Among them, aptamers and antibodies have a high affinity for tumor-derived exosomes. It's significant to note that, in comparison to antibodies, aptamers have superior qualities of chemical stability, ease of synthesis, and affordability. The following problems with conventional electrochemical exosome assays exist, notwithstanding the tremendous evolution that has been attained. First of all, the

immobilization of probes on an electrode surface is a necessary step in these conventional electrochemical assays, which is not only time-consuming and laborious but also inevitably prevents the exosome and probe from being recognized effectively, severely restricting the improvement of the detection sensitivity. Secondly, the capacity of exosomes to be captured could be restricted due to their specific kind of aptamer or antibody recognition. Finally, the majority of these reported electrochemical biosensors only measure the change in the single-peak current signal output, which is susceptible to environmental disturbances that are unavoidable and can lead to false-positive readings.

Limin Yang, Xuehan Yin et al. have created a ratiometric immobilization-free electrochemical sensing technology that allows them to accurately extract tumor exosomes from their intricate biological surroundings and quantify them directly. More importantly, the new method shows good feasibility for clinical sample analysis as it can directly detect tumor exosomes in human serum samples in a complicated sample medium [10].

5. Conclusion

Since lung cancer patients are usually detected at an advanced stage of the disease, which can be a major obstacle to treatment and prognosis, early detection methods are crucial. The limitations of traditional tests, such as their high cost, low detection rate, increased pressure at the patient's lesion, and increased risk of lesions due to invasive and radiological methods, underscore the need for more effective methods to bring the research on early-stage lung cancer detection to maturity. Many clinical trials have demonstrated the promise of biomarkers as an early detection modality in the present. However, further research on biomarkers for lung cancer also requires the help of biosensors, and the application of biomarkers for early lung cancer detection and the development of lung cancer biomarkers are faced with various challenges. This will not only improve early detection rates but also contribute to better treatment outcomes for lung cancer patients in the future.

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The effect of reduced activity of prefrontal cortex neurons on sleep rhythm

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Abstract. The etiology of depression, a prevalent psychiatric disorder, remains elusive. Depression often manifests as a sleep disturbance. A significant proportion of individuals suffering from depression exhibit various sleep rhythm problems. There occurs a well-established and intricate bidirectional association between sleep rhythm abnormalities and depression. The prefrontal cortex (PFC) has been demonstrated to have a crucial role in the development of depression in recent years. The PFC plays a crucial role in the regulation of several cognitive, emotional, behavioral, and other significant activities. The atypical functioning of the PFC might result in the disruption of the sleep cycle. Unusual neuronal activity in the PFC could play a crucial role in controlling both sleep problems and depression. This paper presents a comprehensive overview of the research methods employed in studying the PFC in animal models. It also investigates the potential neural mechanism by which reduced PFC neuron activity may impact sleep rhythm via the PFC-LH-HPA loop. By doing so, this study offers novel insights and concepts that contribute to a deeper comprehension of the underlying causes of depression. Furthermore, this research introduces a prospective approach for addressing depression and sleep disorders through the manipulation of PFC neuron activity, while also highlighting its medical implications and obstacles.

Keywords: PFC, Neuronal activity, Sleep rhythm, Depression

1. Introduction

Depression is classified as a persistent, recurrent, and possibly perilous psychiatric disorder [1]. Depression ranks among the top 10 causes of illness and death globally, as reported by the World Health Organization (WHO). Over 350 million individuals are afflicted by depression. Depression has emerged as the fourth most prevalent global ailment, with a continuous and significant increase in the number of those affected. It is noteworthy that an annual number exceeding 700,000 individuals succumb to suicide as a result of despair. Despite significant advancements in the field of depression research, the precise underlying mechanism of depression remains incompletely understood. There is currently a lack of comprehensive therapy modalities for depression. The pathophysiology and treatment techniques of this condition have consistently been a focus of extensive research. Depression is characterized by symptoms such as a persistent poor mood, lack of interest, weariness, sleep disturbances, along with cognitive deterioration and decreased volitional activity.

Significant symptoms, such as sleep difficulties, not only contribute to the psychological load experienced by patients, but also have a profound impact on their social functioning and overall

quality of life. As per available statistics, a significant proportion of individuals diagnosed with depression exhibit varying levels of sleep disturbances [2]. Several studies have identified variations in circadian rhythms across depressive mouse models, including psychosocial stress-induced depression-like animals [3]. Furthermore, certain investigations have demonstrated that sleep rhythm abnormalities might disrupt molecular expression in the prefrontal cortex (PFC), specifically in relation to other brain regions associated with emotions. This disruption may explain the potential link between sleep disorders and mood disorders [4]. The findings of this study indicate that sleep disturbances are not solely indicative of depression, but rather might play a significant role in its development. There is likely an intricate and reciprocal connection between sleep disturbances and depression.

The PFC plays a crucial role in various cognitive and emotional regulatory processes. Cheng et al. have demonstrated that the PFC plays a significant role in modulating the association between depressive symptoms and sleep quality [5]. This observation implies a potential association between the aberrant functioning of PFC neurons and the development of sleep problems and depression. Hence, the objective of this paper is to examine the impact of diminished PFC neuron activity on sleep patterns and its involvement in the development of depression. Additionally, it aims to suggest potential approaches for managing depression and sleep disorders by modulating PFC neuron activity, while also highlighting its medical implications and obstacles.

2. Sleep disorders and depression

Several psychiatric disorders often coincide with disruptions in sleep and circadian rhythm, leading to significant harm. Sleep has a crucial role in sustaining human existence. Optimal sleep is essential for safeguarding cognitive functions, including learning and memory, as well as for preserving the equilibrium of the body's immune system and metabolism. Issues pertaining to sleep patterns are frequently denoted as sleep disorders.

Depression is commonly associated with atypical sleep patterns, such as early awakening and difficulty initiating sleep. Additionally, patients with depression often experience notable alterations in their overall sleep pattern, including accelerated initiation of the first rapid eye movement (REM) sleep stage and an increase in the duration of total REM sleep [6]. Anomalous sleep patterns have been found to be correlated with heightened levels of despair and anxiety, while extended periods of sleep deprivation have been linked to increased mood fluctuations and suicidal ideation. Do sleep disturbances arise as a consequence of depression or as a contributing factor to it? The feasibility of achieving antidepressant effects in the treatment of sleep disturbances warrants further investigation. Abnormal rapid eye movement (REM) sleep does not directly cause depression. However, the disruption of the sleep structure's homeostasis during non-rapid eye movement (NREM) sleep can lead to abnormal growth of the REM sleep structure, ultimately leading in a decrease in happy feelings. REM sleep inhibition is a mechanism by which certain antidepressant medications exert their effects. According to certain research, sleep deprivation has been found to impair the connectivity between the medial prefrontal cortex (mPFC) and the amygdala, a brain region often involved in emotional processing. In contrast, following emotional stimulation, the activity and connectivity of the mPFC decreased, while the activity in the dorsal ACC and amygdala increased. In addition, the amygdala controls associated functions in the hypothalamus, which is closely linked to the sleep-wake cycle [7].

3. Effects of reduced activity of PFC neurons on sleep rhythm and depression

The PFC is a significant area within the cerebral cortex, located in the anterior portion of the frontal lobe. It can be further split into three subregions: the dorsolateral (dlPFC), ventrolateral (vlPFC), and medial (mPFC) prefrontal cortex. Within the mPFC, it is possible to further categorize it into several regions, including the anterior cingulate cortex, anterior limbic cortex (PrL), and inferior limbic cortex (IL). The PFC is intricately linked to regulatory processes, cognitive decision-making, social interactions, and various other functions.

Studies conducted on patients with depression have demonstrated a general decrease in activity of PFC neurons, as evidenced by basic, clinical, and neuroimaging research [8]. The following aspects mostly demonstrate this phenomenon. In animal models of depression, there is a decrease in the PFC, which leads to neuronal development issues like as synaptic reduction, axon reduction, synaptic density reduction, and neuron reduction in the chronic unforeseeable stress model (CUMS model) [9]. The chronic binding stress model (CRS model) is associated with a decrease in both the length and number of dendritic spines, as well as a reduction in the expression of glutamate receptors [10]. In addition, electron microscopy revealed a decrease in the number of synapses in the dorsolateral prefrontal cortex (dlPFC) among patients with depression [11]. The dlPFC is strongly associated with mood. Furthermore, post-mortem brain study of individuals with depression revealed a substantial reduction in the quantity of neurons and glial cells [12]. The findings indicate that depressed patients exhibit reduced neuronal activity in the PFC, which may be attributed to diminished neuronal function, decreased neuronal firing activity, and reduced synaptic transmission efficiency. The PFC primarily consists of a neuronal network made of Glu ergic pyramidal neurons and GABA ergic pyramidal neurons, which exhibit an excitation-inhibition balance. They disrupt the equilibrium and induce a shift towards inhibition. Furthermore, the cerebral cortex of depressed patients exhibits a notable suppression of Glu signaling, potentially linked to a downward mood. This inhibition is particularly evident in the chronic stress stress model, as chronic stress diminishes the excitability of PFC (particularly mPFC) projection neurons, leading to decreased neuronal firing and compromised neuronal connections. The chronic stress model has the potential to induce depression as a result of the activation of the NLRP3 inflammasome signaling pathway in the PFC [13]. Furthermore, research has demonstrated that stress might interfere with the BDNF-TrkB signaling pathway, leading to a weakness in the subsequent ERK-Akt signaling pathway [14]. Brain-derived neurotrophic factor (BDNF) is a crucial component in the processes of synapse development, neuronal maturation, and synaptic plasticity. Dysfunction of associated pathways results in impairments in the synthesis of proteins at the postsynaptic level, hence exerting a detrimental impact on the maturation and stability of synapses.

EEG investigations conducted on the frontal lobe of mice with CRS depression model demonstrated a significant decrease in the power of all brain waves [15]. The delta, alpha, beta, and other waves exhibit a strong correlation with sleep, and there is a notable alteration in the magnitude of their transmission. The diminished activity of PFC neurons will also impact the sleep rhythm and is intricately linked to the sleep-wake cycle. Furthermore, atypical functioning of PFC neurons can impact neurotransmitters associated with sleep chronology, such as dopamine and norepinephrine. In contrast, an irregular sleep pattern can disturb the properties of PFC neurons, leading to aberrant production of clock molecules and IEG genes in the PFC [16], and reducing the excitability of two thirds of the spinal neurons in the plPFC [17]. The findings revealed a reciprocal relationship between disruptions in sleep patterns and the PFC.

4. Decreased activity of PFC neurons may affect sleep rhythm and lead to depression

The medial prefrontal cortex (mPFC) is a cerebral superior region that governs cognitive processes, attentional processes, and emotional regulation. It extends its neural connections to the hypothalamus brainstem and other brain regions associated with sleep and alertness. The organization mostly consists of Gluergic neurons and GABAergic neurons. mPFC and hippocampus play crucial roles in the development of depression. The involvement of the PFC in the mechanism of consciousness has been a subject of ongoing debate. Its significance extends beyond cognitive and affective activities, as it also seems to play a role in regulating total alertness within the brain. Despite the absence of a universally agreed-upon definition of the PFC across different ethnic groups, it is noteworthy that the mPFC, which encompasses the anterior cingulate gyrus, prelimbic region, and posterior limbic region, has substantial anatomical and functional similarities between rodents and primates. It has been postulated that the involvement of functionally and structurally identical MPFCS in the wakefulness

loop may be attributed to the fact that PFCS project to numerous sleep-related nuclei across various species [18].

The typical sleep cycle consists of two phases: rapid eye movement sleep (REM) and non-REM sleep (NREM). During REM, the activity of the hippocampus theta oscillations is intensified. Research has demonstrated that suppressing pyramidal excitatory neurons in the mPFC brain area can effectively decrease theta oscillation activity during REM sleep and decrease the length of REM sleep [19]. This indicates that inhibitory neurons in the mPFC brain area suppress the incidence of REM. Furthermore, the study observed that excitatory neurons in the mPFC connect to many regions, including the basolateral amygdala, lateral hypothalamus (LH), thalamic nucleus, and lateral tegmental nucleus. Upon the activation of the aforementioned circuits by light, it is seen that only the MPFC-LH loop may facilitate the onset of REM. This suggests that the regulation of sleep-wake by the mPFC is achieved by this particular loop. Moreover, research conducted on rats has demonstrated that injury to the mPFC impacts REM sleep and exacerbates depressive-like behavior [7]. These findings align with prior research indicating that activation of the mPFC facilitates REM sleep, perhaps elucidating the underlying mechanism of sleep rhythm disruption in individuals diagnosed with depression. Nevertheless, these investigations have shown divergent findings, potentially attributable to variations in the precise position of the mPFC.

Several ideas have been proposed to explain the primary causes of depression, such as the monoamine theory, neurotrophic factor hypothesis, HPA axis (hypothalamic-pituitary-adrenal) hypothesis, and neuroinflammation hypothesis, among others. Depression can be attributed to dysfunction in the GABA and Glu transmitter systems. Numerous studies have demonstrated alterations in network functionality inside the brains of individuals diagnosed with depression, with particular emphasis on excitatory Glu neurons and inhibitory GABA interneurons [20]. This phenomenon has the potential to result in diminished signal integrity within the cerebral cortex and hippocampus; however, the precise biochemical pathways behind this phenomenon remain unidentified. Moreover, stress constitutes a significant risk factor for the development of depression, with numerous symptoms of depression being linked to prolonged exposure to stress. In the endocrine system, the HPA axis plays a crucial role. This pathway can be significantly activated by environmental stress and stress events. Research has indicated that individuals diagnosed with depression exhibit heightened activity of the hypothalamic-pituitary-adrenal (HPA) axis. Additionally, a significant proportion of depressed patients experience elevated levels of cortisol, an endogenous glucocorticoid. This heightened cortisol concentration subsequently binds to the intracellular receptor known as GR. However, excessive activation of GR may result in detrimental effects on associated regions [21].

One may hypothesize that the occurrence of chronic stress initially diminishes the functioning of prefrontal cortex (PFC) neurons, resulting in impaired excitatory and inhibitory neurons within the PFC. This disruption of the excitation-inhibition equilibrium, alteration of neurotransmitter levels, and subsequent impact on downstream neurons are potential consequences. The atypical functioning of prefrontal cortex (PFC) neurons will initially impact the mPFC-LH sleep regulation circuit, subsequently influencing the disruption of REM and NREM sleep rhythms. Crucially, neurons in the PFC have the ability to directly or indirectly influence hypothalamic (LH) neurons responsible for generating corticotrophin releasing factor (CRF) via projecting onto LH neurons. The CRF gland plays a significant role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Its primary function is to promote the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. This, in turn, triggers the release of stress hormones, including cortisol, from the adrenal cortex. The persistent secretion of stress hormones can subsequently impact the quality and dynamics of sleep. Conversely, the operational status of the hypothalamic-pituitary-adrenal (HPA) axis can also impact the functionality of the PFC, and an excessively active HPA axis can influence cognitive processes, emotional state, and behavioral patterns. An essential loop for comprehending emergency response and emotional regulation is the neuronal connection between the PFC and the hypothalamic-pituitary-adrenal (HPA) axis. The diminished functioning of PFC neurons impacts the

sleep cycle via the PFC-LH-HPA loop. Prolonged disruption of sleep patterns and anxiety levels additionally impact emotional control, resulting in further disruption of neurotransmitter levels, neuroinflammation, and the release of numerous inflammatory markers. Emotional pathways, such as the prefrontal cortex-amygdala, experience disruption, while the HPA axis becomes excessively stimulated, hence impacting physiological processes related to immunity and metabolism, hormone levels, and finally culminating in the manifestation of depression. Furthermore, the disruption of sleep patterns can also impact cognitive function, resulting in memory and concentration impairments, exacerbating emotional irritation, and intensifying the onset of depression. It is imperative to acknowledge that there could potentially exist additional brain regions or circuits implicated in this hypothesized pathway.

5. Regulation of PFC neuronal activity to achieve antidepressant treatment strategies and challenges

Research has demonstrated that depressed patients exhibit distinct discharge activity in the PFC during acute stress [22]. The discharge activity in the PFC varies between depressed patients during acute stress. This phenomenon may give rise to various therapeutic approaches. The earliest animal trials revealed varying impacts of different depression models, with CUMS models demonstrating a reduction in PFC neuronal activity. In CUMS, sleep patterns are also impacted by exposure to light during the overnight period. Other models of depression, such as the learned helplessness model, exhibit shorter durations. The social failure stress model, which is associated with social disorder, possesses gender and anxiety characteristics that often lead to confusion with depression. The pathological process of the riscepin-induced model is incongruous with depression, as human depression is primarily triggered by prolonged stress and mental burden. Consequently, this model is somewhat less dependable [23]. These depression models fail to account for the transient nature of depression. Attempting to modulate the functioning of PFC neurons to combat depression can only be initiated from the chronic stress model. However, it is not feasible to treat all patients without a more comprehensive and effective depression model, as well as a more pragmatic depression assessment strategy.

The existing experimental technology exhibits limitations in its ability to regulate PFC neuronal activity. Various methods, including electrophysiology, transcranial magnetic stimulation (TMS), optogenetics, and transgenic technology, are unable to effectively augment neuronal activity in specific brain regions. Several studies have provided evidence that the introduction of light-activated channel proteins in the mPFC can lead to an optogenetic stimulation of mPFC discharge. This optogenetic intervention has been shown to have a significant antidepressant effect, while not compromising general motor activities, anxious behaviors, social memory, and other related functions. These findings highlight the potential of optogenetics as a viable approach for enhancing neuronal activity as a treatment strategy for depression [24]. While the current research is mostly centered in optogenetics, its applicability in clinical trials has yet to be evaluated. The utilization of transcranial magnetic stimulation (TMS) has demonstrated enhanced efficacy in promoting neuronal activity, particularly in the context of clinical application for depression treatment. Specific brain regions are stimulated in order to reduce symptoms of depression. This approach is currently regarded as a generally safe strategy with little side effects. However, the therapeutic efficacy of this strategy is not readily apparent [25]. Depending on the specific circumstances, the current utilization of monoamines remains higher. Overall, TMS exhibits superior research value. Furthermore, at present, there are no pharmaceutical substances that facilitate neuronal stimulation in certain areas of the brain, and medications are more susceptible to adverse reactions and long-term reliance. Regardless of the methodology employed, it is imperative to consider the disparities in the kinds, arrangement, and composition of PFC neurons between the human PFC and those of mice or other animals when converting animal investigations into clinical applications. The mPFC, which encompasses a significant amount of the brain's PFC area, has a greater abundance of neuronal subtypes and synaptic connections. It assumes a crucial function in higher cognitive processes and emotional regulation,

hence proportionally enhancing its significance [26]. Due to its inherent complexity, the application of this concept necessitates a more cautious approach.

6. Conclusions

All things considered, there is strong evidence that aberrant PFC neuronal activity is important for the circuits linked to depression and sleep patterns, probably in both directions. This paper examines the correlation between sleep and depression, as well as the impact of abnormalities in PFC neurons on sleep and performance resembling depression. Based on a synthesis of existing data, it is hypothesized that prolonged stress leads to a reduction in the functioning of PFC neurons. This drop in activity subsequently impacts the PFC-LH-HPA circuit, resulting in an irregular sleep pattern and ultimately causing depression. Indeed, additional investigation is required in order to elucidate the sequence, such as to what extent does stress contribute to diminished neural activity? What is the initial pathway that becomes activated? What are the other behaviors that are influenced by PFC projection? Furthermore, it is imperative to take into account the impact of additional neural circuits, such as the amygdala and the hippocampus, on behavior, cognition, and emotion. These studies aim to elucidate the atypical functioning of PFC neurons in mood disorders and its association with the sleep circuitry. This will facilitate the advancement of therapeutic procedures and strategies that are more efficacious, secure, and tailored to specific needs.

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A comparative study of Chinese and foreign research relevant to the drift diffusion model in the last decade

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Abstract. This study evaluates the differential research approaches of Chinese and international scholars regarding the drift diffusion model in mental processes through a literature review from the last decade. The application of DDM theory in cognitive research varies between Chinese and foreign study methodologies. The differences in decision-making mechanisms among cultural groups result in this outcome. Inconsistency has been identified in international academic research. They cover reward rate optimization, decision thresholds, decision time, and more. On the contrary, advancements in Chinese studies involve the development of innovative models. They are Bayesian and semi-parametric. These are designed for mixtures combining drift-diffusion processes and the inverse Gaussian distribution. In addition, they encompass elucidating the neural processes and implementing DDM techniques. This use of DDM reveals the rewarding perception and decision-making linked to depression. It also reveals the brain patterns of social anxiety. These comparisons aim to summarize the differences between Chinese and foreign studies. They also analyze them. It will find the causes of these differences. Moreover, it will determine how Chinese clinical care can use them. The DDM studies show its importance in decision-making patterns. Scholars have the opportunity to apply these tools in a variety of scientific fields, including neuroscience, psychology, artificial intelligence, and cognitive research.

Keywords: Drift-Diffusion Model, Comparative study, Culture Difference

1. Introduction

The perceptual decision model aims to understand how animals detect, differentiate, perceive, and classify information [1]. It occupies an important place in neuroscience and psychology, showcasing the benefits of integrating mathematical and biological approaches to studying the brain and its functioning [1].

The drift-diffusion model is widely recognized and utilized in academia because of its extensive data collection capabilities, particularly in the last decade, where it has garnered interest from researchers worldwide for its integration with psychological research and novel ideas [2].

As a simple model of the cognitive process of bi-optional decision-making (apply only to fast bi-optional and single-stage decision-making processes), the DDM is an assumption that noise in the decision process is observable and that the line of the picture always goes from a starting point to one of two response criteria or boundaries, with the starting point labeled z and the boundaries labeled a and 0 . The rate at which the information accumulates is called the drift rate (v) and is determined by the amount of information extracted from the stimulus quality of the information. In some research, the values of drift rate v are different for each stimulus condition because they differ in difficulty [3]. The analysis allowed measurement of the magnitude of noise in the accumulator's memory [4].

The purpose of this paper is to summarize and analyze the results of DDM research in China over the past decade, to compare the differences between Chinese and foreign research, to analyze the possible reasons for the gap, and to try to assess the possibility of its use for clinical interventions in China.

2. Literature review of Chinese and foreign studies related to diffusion drift modeling in the last decade

2.1. Foreign studies related to diffusion drift modeling in the last decade

A study of reward rate optimization in two-choice decision-making explores empirical tests of theoretical predictions [5]. A drift-diffusion model (DDM) implements an optimal decision procedure for a static, two-choice forced-choice task [6]. The height of the decision threshold applied to the accumulation of information on each trial determines the speed-accuracy trade-off (SAT) of the DDM, a finding that explains the ubiquitous character of human performance in rapid-response tasks [7]. Another study by this team investigated a missing link-filling approach linking decision-making to the operant conditioning literature and extending choice proportion prediction to inter-response time prediction [8]. The approach was implemented in an adaptive version of the Drift-Diffusion Model (DDM), which is widely used in decision-making research to explain reaction time distributions [9].

The pacemaker-accumulator (PA) system has been an extremely popular timing model in the 50 years since its introduction by Treisman [10]. Although the dominant PA model during this period - Gibbon and Church's scalar expectation theory (SET) - invokes most of its assumptions, several alternative timing models based on different assumptions have been devised [11]. Like Treisman's model, this time-adaptive, opposing Poisson, drift-diffusion model need not assuming Weber's law in the first place, it is possible to take into account timescale invariance [12]. A preliminary introduction to a hierarchical extension of the Drift-Diffusion Model (DDM) is given [13]. This formal decision-making model is commonly used in cognitive science but has been little used in social and personality research in Western academia [14].

Revealed preference is the primary method for inferring preferences, but is limited in that it can only rely on discrete choice data [15]. When a person chooses one choice over another [16], it is predicted how likely they are to make the same choice again [17]. Linking the two main sub-components of internalization - antagonism and inhibition - to specific control processes through a series of inhibitory control tasks and corresponding computational modeling [17]. used a hierarchical drift-diffusion model (DDM) to fit participants' task behavior, partitioning their decisions into a series of specific cognitive processes [17]. Fisher proposed a model that describes how attentional fluctuations to choose set features affect decision-making [18]. One of his studies found that the Attentional Drift-Diffusion model accurately describes choice, reaction time, and how these variables relate to visual attention to attributes and options [18]. Karimi proposed a computational method to quantify decision-making ability in patients with mild cognitive impairment and attention deficit [19]. He also developed a classification model for detecting cognitive impairment based on the estimated parameters of a drift-diffusion model [19].

2.2. Chinese studies related to diffusion drift modeling in the last decade

The main results of cognitive research on drift-diffusion modeling (DDM) in China include:

(1) Developed a novel Bayesian semi-parametric inverse Gaussian drift-diffusion mixture model for multi-choice decision-making processes [20];

(2) Describing the neuron cognitive mechanisms underlying attentional bias to pain using a hierarchical DDM [21];

(3) Applying the DDM to reveal impaired reward-based perceptual decision-making processes associated with depression in girls in late childhood and early adolescence [22].

This study focuses on assessing subject-level heterogeneity in parameter trajectories and demonstrates how a stratified version of the DDM can advance the study of mental processes. In addition, this study states that adults with depression are deficient in accumulating reward-based evidence and quantifies the rate of evidence accumulation and starting point bias in depressed individuals using a stratified DDM [23].

(4) Applying the DDM to reveal the Specificity of different components of emotional processing of social anxiety associated with the fitted parameters of emotional processing [24].

This study recruited 31 university students with an average age of 21.97 to judge whether the lighted walker expressed half of the emotions of anger and happiness and half of sadness and neutrality in an emotional movement recognition task, and recorded accuracy and reaction time, then fitted the behavioral data using a drift-diffusion model (DDM) and evaluated parameters including the non-decision time and the non-decision time, thus achieving the goal of analyzing the relationship between social anxiety and other emotional processes. Decision time) and the non-decision time) to reach the goal of analyzing the relationship between social anxiety and other emotional processing [25].

(5) Incorporating eye gaze to describe individual decision-making behavior models [26].

This study examined the relationship between gaze and decision-making in value-based decision-making using a gaze-based manipulation paradigm and found that manipulating the amount of time, subjects spent looking at options interfered with the choice process, that gaze manipulation primarily affected the late time course of decision-making, and that model parameter estimates were more supportive of proximate cause models. The results of the study show the reliability and validity of the proximate cause hypothesis of the drift-diffusion model [26].

(6) Analyzing the ongoing impact of errors on future decision-making [27].

Results show a relationship between response time on the first trial after an error and the average accuracy on subsequent trials [27]. This study contributes to the understanding of the dynamic decision-making process and addresses the limitations of traditional approaches by developing a multi-category drift-diffusion model with independent latent processes for each decision category [27].

3. DDM research areas internal micro-analysis

3.1. The speed and accuracy of research of DDM

In research on the problem of choosing when to choose, the ongoing motivational state of an animal when making a perceptual decision is the central issue [28].

In terms of the speed and accuracy of perceptual decision-making, how this can be achieved at the neural level in both human and non-human subjects has been the subject of recent research. Recent studies have begun to focus on the neural mechanisms by which this is achieved in human and nonhuman subjects. For example, when monkeys are compensated in favor of speed over accuracy, the selection moves more quickly toward the edge. The accuracy of the noisy decision signal is progressively improved during successive sampling, with high bounds leading to slower but more accurate decisions and low bounds reducing consideration time at the expense of performance. Subsequently, the limit level is finally determined by measuring rate and accuracy [28].

Since the accuracy of the uplink selection signal increases bit by bit during successive tests, high bounds lead to slower but more accurate selections, whereas low bounds reduce the time to think, thus affecting the execution. The termination rate in the LIP is linked to an execution similar to that computed by the DDM by encoding the value of the collective selection variable [29]. A common,

signal-independent level of neural signaling before eye movements have been shown to support this hypothesis [30]. Although recordings from the FEF, LIP, premotor cortex (PMd), and basic engine cortex (M1) suggested a delineation of results, they reached certain arrangements, such as the unfinished hypothesis of unpredictable content and limited aggregation by the DDM [30].

Meanwhile, in psycho-physical research, the experimenter can decide to control the quality of tactile symbols, either in blocks or previews. In the former case, the winning rate can be improved by applying appropriate constraints, e.g., modules whose level remains stable in each preview within a block [31].

Furthermore, the groundbreaking idea is that the selection could be driven by a strong, unproven quantity called the ‘limit’. Selection could be driven by a powerful, unproven quantity known as the “earnest” signal, which completes a descending limit from nothing, blowing up the later collector state [31].

3.2. Balancing perceptual choices based on likelihood and value in DDM research

This area of related research aims to explore the control of perceptual choices through likelihood and value pairs to discover a wide range of strategies that utilize different modeling frameworks. How do humans and monkeys combine seething tangible evidence with encoded correlational data at the social and brain levels to select the likelihood or value of a situation to make an optimal choice [32]?

In addition, DDM-related research focuses on how humans and monkeys combine encoded contextual information with noisy sensory evidence at the behavioral and neural levels to make optimal decisions [32]. One crucial balance is known as LIP, which emphasizes the adjustment of perceptual choices to boosted values or probabilities [32].

3.3. DDM and Decision Confidence

For humans, perceptual orientation is an area of strength determined by certainty or vulnerability, which can be assessed when asked to report on our ‘certainty’; and cross-species DDM studies with rodents, monkeys, and humans have begun to show signs of neuron variability in decision confidence [33].

People can explicitly report certainty on scales, and clinicians refer to this type of computation as “brain computation.” In “metacognition,” how certainty is encoded in brain circuits requires a rational definition. Certainty is understood in terms of how the selected data are read off the decision-referenced representations and preserved in the accuracy-referenced margins (the likelihood of being correct is uniquely associated with the alternating arrangement of brain circuits [33].

4. Comparison

In terms of research content and application methods, the similarities and differences between the Chinese and Western uses of DDM are as follows:

(1) The application of the Drift Diffusion Model (DDM) in cognitive research shows the cultural differences in cognitive processes between China and other countries [34]. For example, one study has shown that Chinese and Danish people have very different neural activities and decision-making strategies when applying the drift-diffusion model [34].

(2) One study examined the differences in cognitive mechanisms between cultural groups by comparing DDM parameters, and the results showed that Chinese dependencies were at a higher distance compared to English, thus reflecting the higher syntactic complexity of Chinese texts [35].

(3) The similarity between Chinese and Western DDM studies lies in the fact that the application of DDM in cognitive science aims at exploring and explaining the nuances of cognition in different cultures and contexts, and they both may select subjects for testing in their respective cultural contexts [36]. Moreover, in the applied research of DDM, both Chinese and foreign countries focus more on artificial intelligence cognitive research [36].

5. Conclusion

In DDM research, the differences between Chinese and foreign academic research are reflected in many aspects, which not only reflect the different dimensions and different orientations in DDM modelling research, but also are mainly reflected in the differences in academic tradition and culture. China and the West in the literature of the last decade reflect obvious differences in the focus, research methods and evaluation criteria of DDM issues.

Meanwhile, this difference also demonstrates different academic values and research styles in different academic and cultural contexts. China has had a more exciting performance in the use of mental science in the last decade, while Western academics have focused more on basic research itself.

6. Discussion

Through the analysis of comparisons and conclusions, the importance of incorporating the drift-diffusion model into decision-making research is evident, pointing towards the potential integration of this model with advancements in psychological research over the past ten years. The overall success of the DDM research also shows the pivotal position of the decision modeling type of research in neuroscience and psychology, as well as the role of this research in the cognitive research of AI, which will have a direct and profound effect on the productivity development of society. Some of the research integrated with the humanities has also shown the potential of the model to be integrated with psycho-social and therapeutic models, especially in China. In addition, DDM research also transcends the theoretical schools of clinical treatment from the dimension of brain science evidence, and it provides a new line of research that will provide more evidence-based evidence for the various original clinical psychotherapy techniques.

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Hydrogel dressing in diabetic wound healing

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Abstract. With the intensification of social ageing, the number of geriatric patients continues to increase. Among them, the problem of diabetic patients whose wounds cannot heal for a long time and even cause amputation has received widespread attention. Excessive inflammation caused by adverse exogenous and endogenous factors that interfere with the regulation of wound inflammation is the main factor in the failure of the wound to heal according to standard procedures. As a polymer with a three-dimensional structure, the hydrogel is expected to be a new type of chronic wound dressing with good potential due to its good biocompatibility and drug loading. Summarizing the advantages of new functional hydrogel dressings and the progress of research in diabetic wounds, it is expected to provide some new ideas for clinical research and functional improvement of hydrogels.

Keywords: hydrogel, diabetes, wound healing, anti-inflammatory

1. Introduction

As the largest organ and outermost layer of tissue in the human body, the skin is the main barrier against external environmental stimuli [1]. Therefore, research on the rapid healing and regeneration of injured skin is of great significance in effectively improving the quality of life of patients and reducing wound pain. In some cases, the appearance of acute inflammation caused by a bacterial infection or other factors causes chronic wounds not to be repaired in a timely and orderly manner according to the normal healing stage, which poses a great challenge to the clinical treatment of chronic wounds such as diabetes [2]. Therefore, the development of hydrogel dressings combined with novel antibacterial and anti-inflammatory technologies has become a key means to solve the problems of wound healing and skin repair.

2. Problem of wound healing

2.1. Wound healing stage

Wound healing is a complex physiological process, which is generally divided into four stages - hemostasis, inflammation, proliferation and remodeling [3], and the orderly progress of each stage can ensure wound healing.

As soon as the skin is damaged, the exposed collagen triggers an intrinsic and extrinsic clotting cascade [4]. The release of coagulation factors can stimulate fibrin matrix deposition, platelet aggregation and trigger vasoconstriction to reduce blood loss, and after 5-10 minutes of vasoconstriction, platelets and leukocytes migrate into the temporary matrix. Platelets release chemical signals that allow

white blood cells to migrate from blood vessels in the direction of injury. Platelets kill bacteria by activating the clotting cascade, which stimulates the wound to produce inflammatory cells. In addition, platelets secrete proteins such as thrombospondin and growth factors such as insulin growth factor (IGF), platelet-derived growth factor (PDGF), and transforming growth factors (TGF- α , TGF- β) to assist in the wound healing phase after hemostasis [5]. The early stages of inflammation involve the activation of the innate immune system, and locally released growth factors (GFs) and cellular mediators recruit inflammatory cells such as neutrophils and monocytes to the wound site, inducing the activation of intracellular signaling pathways, resulting in the expression of a large number of genes such as cytokines, chemokines, and antimicrobial peptides to initiate and maintain the inflammatory response, thereby achieving the effect of removing foreign bodies, bacteria, and harmful endogenous tissues. In the later stages of inflammation, macrophages produce cytokines and growth factors that help fibroblasts and endothelial cells proliferate and begin to enter the proliferative phase. The proliferative stages include granulation tissue formation, vascular regeneration, and reepithelialization. Granulation tissue is composed of a large number of fibroblasts, granulocytes, macrophages, and blood vessels, which partially restore the structure and function of the injured skin. Fibroblasts play a central role in the formation of granulation tissue, fibroblasts proliferate in large quantities through mitosis, synthesize and secrete a large number of collagen fibers and matrix components from 4~5 or 6 days, and form granulation tissue together with new capillaries, fill wound tissue defects, and create conditions for the coverage of epidermal cells [6]. During the proliferative phase, new blood vessels are formed, and the synthesis of enhanced collagen fibers begins. Epithelial cells, fibroblasts, and keratinocytes build granulation tissue. Complete repair of skin wounds can take weeks or even more than a year. The exact duration depends on the patient's age and health.

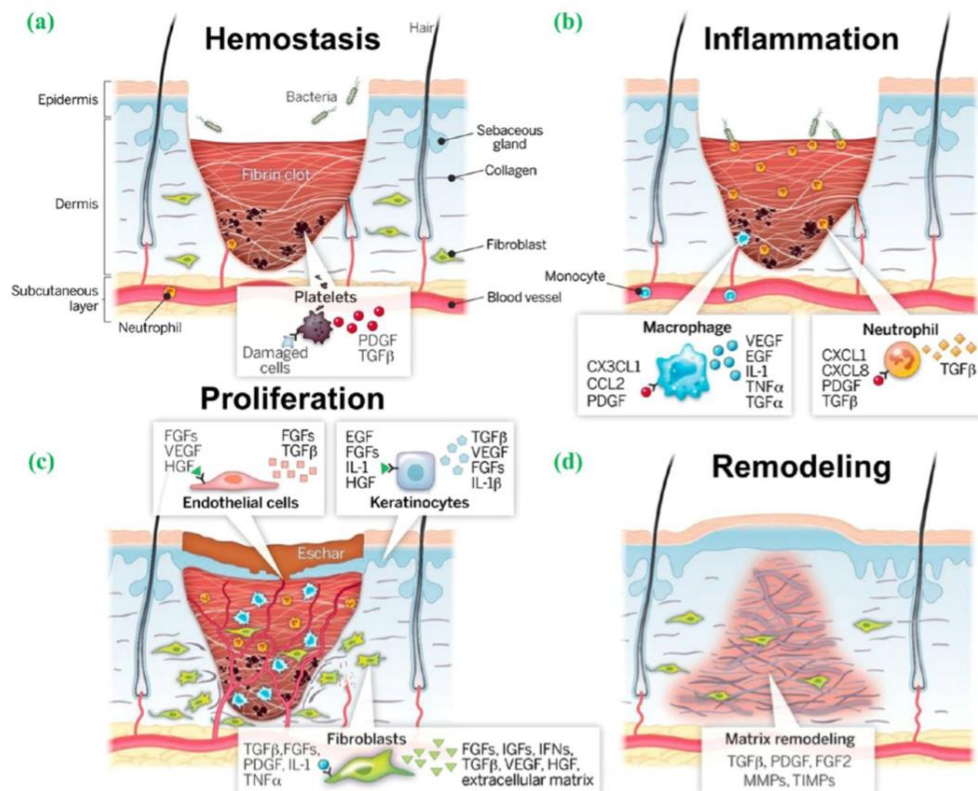


Figure 1. Four stages in wound healing: (a) hemostasis, (b) inflammation, (c) proliferation, and (d) remodeling [7].

2.2. *Chronic wounds*

According to the nature of wound healing, it can be clinically divided into acute wounds and chronic wounds. Acute wounds are usually caused by mechanical or chemical injuries or burns, and the wound can heal completely, usually in 8-12 weeks. Chronic wounds are wounds that are caused by various pathological factors due to the presence of an underlying disease, deviate from the normal healing process of the wound in some way, and stagnate at a certain stage [8]. Chronic wounds have a high bacterial biofilm loading rate, with defects such as persistent inflammatory phases, excessive proteolysis, etc. Slow formation of new blood vessels, impaired angiogenesis, and inadequate supply of oxygen and nutrients to the wound site further delay the advancement of the wound healing process. Chronic wounds do not heal after 12 weeks and have no tendency to heal.

Studies have shown that the main causes of impaired healing of diabetic foot ulcers (DFU) [9] include vascular lesions, neuropathy and infection [10]. Hyperglycemia and abnormal glucose metabolism in diabetic patients lead to vascular-related diseases that affect the distribution of distal vessels emanating from the femoral artery, arch of the foot artery, and metatarsal artery. When the distal artery is unable to efficiently distribute oxygen and nutrients to the wound, defects in microcirculation, decreased capillary size, hardening of the basement membrane, and defects in arterioles occur. In addition, endothelial cell dysfunction can occur, reducing basic physiological activity, including nitric oxide synthase, and the inability of arteries and arterioles to dilate, resulting in abnormal blood flow and poor wound healing. In addition, diabetes mellitus can cause neuropathy, impairing the function of motor, sensory and autonomic nerve fibers, reducing their ability to detect pressure, and causing nerves to be insensitive to external heat, pressure and other stimuli from the wound. As a result, people with diabetes experience repetitive foot trauma that leads to poor wound healing. Besides, hyperglycemia [11] is the main source of infection, because high blood glucose concentration can inhibit the phagocytosis of white blood cells, reducing the ability to prevent infection, and the high sugar environment is a good medium for bacteria, so bacterial infections such as *Escherichia coli* and *pneumococcus* are extremely common in diabetic patients. When the body's resistance is weakened and a wound appears, bacteria will take advantage of the weakness and cause wound infection. Abnormal glycemic control can lead to immune dysfunction in the body, impair white blood cell activity and complement function, and accelerate the development of tissue infection. Therefore, diabetic patients are susceptible to infection and not easy to heal.

As a result, the wound healing process in patients with hyperglycemia stalls at any of the four stages of normal wound healing, eventually manifesting as long-term symptoms of inflammation.

2.3. *Treatment of diabetic foot ulcers*

In modern life, a variety of methods have been shown to promote the healing of DFU and reduce the incidence of amputation, including local wound glycemic control, debridement of the wound site, foot decompression, hyperbaric oxygen therapy, electrical stimulation, negative pressure wound therapy, topical use of growth factors and dressings, etc.

Glycemic control: Abnormal blood glucose levels are a major cause of several complications in patients with diabetes and an important factor in impaired healing of DFUs. When the patient's blood glucose level > 11.1 mmol/L, neutrophil function is significantly impaired. At the same time, high blood sugar levels increase the rate of wound infection in patients. It also increases the incidence of peripheral arterial disease. Therefore, effective control of the patient's glycemic index is essential for the healing of DFU.

Wound debridement: Through debridement, necrotic tissue, protein fragments and infectious substances can be removed from the wound surface, effectively promoting the production of granulation tissue, reducing the number of wound infection bacteria, and stimulating the secretion of local growth factors to improve wound healing. Existing debridement methods include surgical debridement (performed by scalpel, tissue forceps, bending scissors, etc.), autolytic debridement (artificially creating a moist wound environment to activate the host's autonomic defense mechanisms), and enzymatic debridement (collagenase, papain, and dextran derived from animals such as crabs).

Topical use of growth factors: Studies have shown that the addition of growth factors (such as insulin-like growth factor, fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor) to dressings can effectively stimulate cell mitosis, improve cell chemotaxis, and accelerate wound healing. However, it should be noted that some growth factors are carcinogenic.

Dressings: Ideally, dressings for wound healing in DFU should have functions such as maintaining water balance at the wound site, stimulating the production of self-growth factors, antibacterial, and oxygen permeability. In addition, therapeutic dressings containing drugs should have a controlled drug release capacity. All things considered, the hydrogel is undoubtedly the most advantageous new wound dressing at this stage.

3. New wound dressing – hydrogel

3.1. Development of hydrogels

Traditional wound dressings mainly include gauze, bandages and foam dressings. Although these wound dressings have been successfully used in clinical practice for a long time, these traditional dressings have many defects, such as poor antibacterial effect, which make the traditional dressings not achieve the expected therapeutic effect. Therefore, the development of a novel dressing that promotes chronic wound healing has attracted great attention.

First of all, the hydrogel has a high-water content, which can provide the moist environment needed for wound healing while achieving the pain of cold compresses. Secondly, the hydrogel has good biocompatibility, is non-toxic and biodegradable, is safe to use, and will not cause rejection reactions such as hypersensitivity and inflammation. In addition, the three-dimensional network cross-linking structure of the hydrogel does not affect the gas exchange and normal respiration of cell tissues, which is conducive to the repair, growth and reproduction of cells at the wound. Its special structure can also be used as a carrier of functional materials, containing a variety of drug molecules that are beneficial to wound repair, preventing them from dissolving quickly, and controlling their continuous and efficient release at the target site.

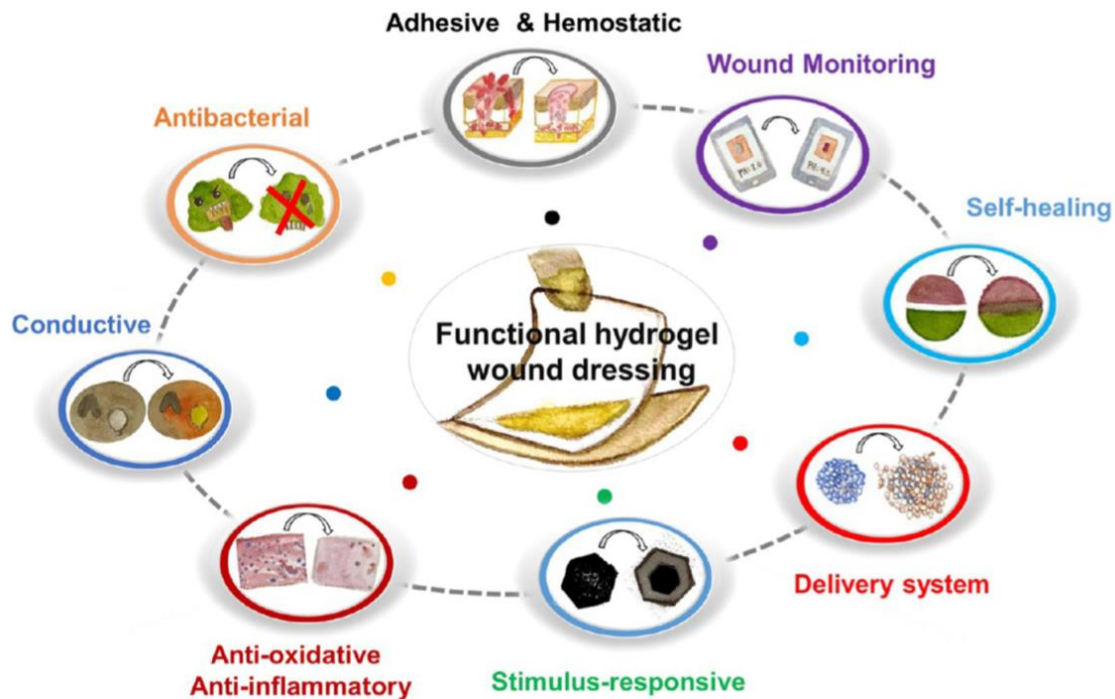


Figure 2. Advanced functions appeared in hydrogel wound dressing [12].

3.2. *The progress of antibacterial hydrogel*

The development of antimicrobial agents combined with hydrogels as wound dressings is very promising. As research has evolved, many antimicrobial hydrogels have been developed, such as, nanoparticle hydrogels with metal ions (silver, copper, gold); metal oxide (zinc oxide, titanium dioxide, nickel oxide) nanoparticle hydrogel; Hydrogels containing antibiotics (ciprofloxacin, gentamicin, vancomycin); Bio-extracts (honey, curcumin, vitamin E) encapsulated hydrogels; Hydrogels containing antimicrobial polymers (chitosan, polylysine, polyethyleneamine); hydrogel containing antimicrobial peptides; Carbon materials (graphene oxide) are coated with hydrogels, etc. [13]. However, some antimicrobial materials have various defects due to their inherent properties, such as, if too much metal nanoparticles are used, they may cause symptoms such as dermatitis, redness, swelling, itching and ulceration of the wound skin; Long-term misuse of antibiotics has led to the emergence of drug-resistant bacteria (methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci) [14] and some intractable superbugs, which are resistant to a variety of antibiotics and seriously hinder wound repair and skin regeneration; Although bioextracts are non-toxic, they are clinically deficient due to their low water solubility, low stability, and poor bioavailability.

At this stage, the method of using photothermal technology to achieve an antibacterial effect is extremely advantageous. Photothermal therapy is mainly used for the treatment of a variety of common tumors, and the exploration of its antibacterial properties is still in the initial stage of research, and has great potential for development in the field of treating chronic skin wounds. The principle of photothermal antimicrobial technology is the photothermal effect, which usually uses near-infrared light to irradiate the surface of objects where bacteria are present, and uses appropriate photothermal agents to convert light energy into local physical heat, taking advantage of the sensitivity of bacteria to high temperatures, resulting in the death of bacteria by heat [15]. Through the efforts of researchers, multifunctional hydrogels with a variety of photothermal properties have been developed.

Zhou et al. [16] introduced gold nanoparticles into the mesoporous structure of mesoporous polydopamine and modified the hydrogel. Experiments have proved that the presence of hydrogels greatly reduces the toxicity of gold nanoparticles to the skin, making them have long-term biocompatibility. The combined use of two photothermal agents, mesoporous polydopamine and gold nanoparticles, results in an enhanced photothermal effect (approximately 70°C). Moreover, the hydrogel has excellent reproducible photothermal antibacterial ability. However, high temperatures can cause damage to the healthy tissue cells around the wound. Therefore, it is of great significance to reduce the effective antimicrobial temperature of PTT. Qiao et al. [17] prepared a self-healing hydrogel with certain photothermal stability, in which Fe³⁺ oxidized dopamine and polydopamine-encapsulated poly(thiophene-3-acetic acid) were added as active ingredients to stably increase the temperature by 23°C. After 10 minutes of near-infrared exposure, the survival rate of both *E. coli* and methicillin-resistant *Staphylococcus aureus* was less than 0.0001%. Experiments have proved that the combined application of photothermal antimicrobial technology and low concentration of antimicrobial agents can produce better antibacterial effect in hydrogel dressings while reducing the damage caused to the body. Although Qiao et al. [17] effectively reduced the temperature of PTT antimicrobial action, it still did not meet the ideal range (below 50°C) to prevent normal tissues from being destroyed by high temperatures. Zhao et al. used glycocalyx to simulate the function of phenylboronic acid to make the hydrogel specifically capture the bacteria on the surface of the hydrogel and destroy the bacterial membrane, which enhanced the sensitivity of bacteria to PTT, so that the hydrogel could achieve a good antibacterial effect at a temperature lower than 49.6°C. In addition, the use of non-covalent hydrogen bonding of polydopamine (PDA) with carboxymethyl chitosan and polyacrylamide (Fig. 3), the highly dynamic reversible borate covalent bonding of PDA to phenylboronic acid-functionalized graphene, and the stacking of PDA give the hydrogels good mechanical properties and self-healing ability.

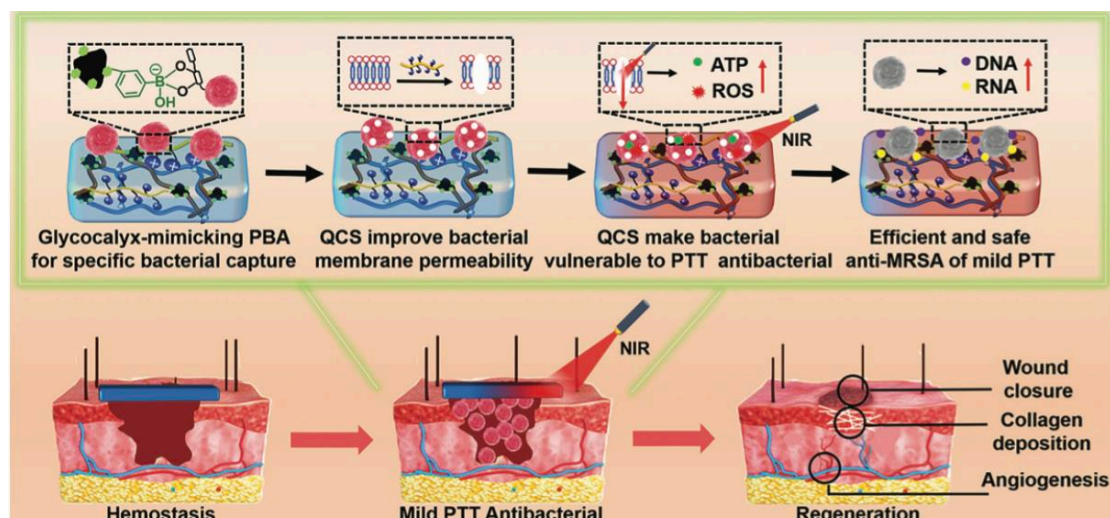


Figure 3. [17] Schematic diagram of photothermal antimicrobial repair performance of multifunctional hydrogel dressing First, glycocalyx mimicking phenylboronic acid can capture methicillin-resistant *Staphylococcus aureus* on the surface of hydrogels. The carboxymethyl chitosan will then disrupt the membrane of the trapped bacteria and achieve an effective antimicrobial effect through a mild photothermal effect at low heat (below 50°C) coordinated by photothermal 3-aminophenylboronic acid modified reduced graphene oxide (rGB). Biocompatible hydrogels accelerate tissue regeneration and healing of infected wounds.

3.3. Limitations of enhanced mechanical properties of hydrogels

It is important to note that the rupture of the hydrogel due to mechanical stretching significantly increases the risk of infection by external bacteria. Consequently, the quest for effective methods to enhance the mechanical strength of hydrogels while preserving their structural integrity has garnered significant interest within the scientific community. In recent years, self-healing hydrogels have been developed. As a new type of smart material, self-healing hydrogel has better mechanical properties than traditional hydrogels, and in addition, hydrogels have achieved self-healing functions and structural damage functions due to their dynamic and reversible three-dimensional polymer network structure prepared by physical or chemical cross-linking [18].

So far, researchers have developed multiple types of hydrogel dressings with different properties, and the current stage of hydrogel dressings has shown injectability, strong adhesive properties, good self-healing ability, efficient antibacterial ability, antioxidant capacity and drug release properties.

According to the healing mechanism, self-healing hydrogels can be divided into physical self-healing hydrogels and chemical self-healing hydrogels. Physical self-healing hydrogels reconstruct networks between molecules, oligomers, or polymer chains by forming dynamic non-covalent interactions [19] (e.g., hydrophobic interactions, host-guest interactions, hydrogen bonding, crystallization, metal-ligand synergies, etc.). Chemical self-healing hydrogels rebuild the network by forming dynamic covalent bonds such as hydrazide, phenylborate, disulfide, imine, metal-ligand coordination, reversible radical reaction, and reversible Diels-Alder cycloaddition reaction. Among them, Schiff base bonds, imine bonds, are widely used in self-healing hydrogels, and the structure is formed by the reversible condensation reaction of primary amines and active carbonyl groups [19] (such as aldehyde groups and ketone groups). Schiff base bonds are one of the few dynamic cobonds that can be formed without stimulation, and dynamic equilibrium can be achieved in the presence of water molecules. In addition, aromatic Schiff bonds are more widely used than aliphatic Schiff bonds due to their more stable structure.

Huang et al. [20] developed a self-healing hydrogel based on water-soluble carboxymethyl chitosan (CMC) and rigid dialdehyde-modified cellulose nanocrystals (DACNCs). Through dynamic Schiff bond cross-linking between CMC and DACNC, the hydrogel is self-healing in less than 5 minutes without external force. In addition, it has been found that the mechanical properties of hydrogels can be

effectively improved by adjusting their concentration and crosslink density. Zhang et al. [21] introduced adipic acid, diacylhydrazide and calcium ion (Ca^{2+}) into quaternary ammonium, carboxymethyl chitosan and aldehyde-modified hyaluronic acid to prepare a novel hydrogel. Through the synergistic effect of imine bonds, hydrazone bonds and coordination bonds, the mechanical properties and self-healing properties of hydrogels were significantly improved. Experiments have shown that the hydrogel has a compressive stress of up to 896.30 kPa. In addition to chitosan, other amino polymers, such as gelatin, can also be used to form imine bonds with aldehyde functionalized polymers to prepare dynamic hydrogels, which proves the versatility of imine bonds in practical applications.

Due to its high mechanical strength and self-healing ability, the self-healing hydrogel successfully ensures its structural integrity, making it less prone to tearing, reducing the risk of wound exposure and reinfection to a large extent, and avoiding further damage to the wound. It is worth noting that the hydrogel formed based on the dynamic Schiff base bond can be painless removal by using amino acid solution and reduce the pain of the patient when changing dressings [18], which is an advantage that other hydrogels do not have. However, it should be noted that as the crosslinking density increases [17], the pore size of the hydrogel decreases significantly, which reduces the swelling rate of the hydrogel, limits its ability to be used for drug delivery, and adversely affects cell growth.

4. Conclusion

In order to avoid the toxic effect of hydrogel dressings on the skin, most of the research teams adopted the strategy of using natural materials as the main body and modifying them to prepare hydrogels, which also achieved certain results in enhancing the antibacterial effect, shortening the wound healing time and reducing the pain of dressing change. However, the hydrogels on the market still cannot meet the various clinical needs for wound healing. In order to cope with the problems of insufficient toughness of hydrogels and long repair time after rupture, we can try to develop bilayer hydrogels with strong and weak bond layers in the future. The strong bond layer adopts a high-toughness structure, which is not easy to break, which limits the activity of the human wound to a certain extent and avoids wound rupture. The weak bond layer adopts a structure with self-healing properties to ensure the repair of the hydrogel after fracture. When stretched, the strong bond layer does not break, maintaining the integrity of the hydrogel, and the breakage of the weak bond layer improves the mechanical properties of the hydrogel, thereby reducing the risk of microbial infection during wound exposure. Studies have shown that mild stimulation at temperatures between 40-43°C can promote angiogenesis, and mild thermal effects should promote type I collagen deposition and neurovascular reintegration, aiding wound healing [22]. Therefore, in addition to high-temperature antimicrobial treatment using the photothermal effect, skin repair can also be carried out with gentle PTT. In the future, we can develop temperature-adjustable hydrogels that can achieve different effects of sterilization and skin cell regeneration at different stages of wound treatment.

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Research progress of photodynamic therapy in the anti-tumor field

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Abstract. Photodynamic therapy (PDT), which is based on activating photosensitizers by light of a certain wavelength to produce anti-tumor effects, has attracted much attention because of its non-invasiveness and high selectivity for tumors. In many tumor diseases with high mortality rates, PDT has achieved better therapeutic effects when combined with a variety of therapies and has become one of the most important methods for treating a variety of cancers. In this review, we have collected research on the application of PDT in cancer and its combination with chemotherapy, immunotherapy, and nanomaterials in recent years, aiming to show that the combination of PDT with other therapies deserves further exploration

Keywords: Photodynamic therapy, Photosensitizer, Anti-tumor, Synergistic treatment

1. Introduction

Photodynamic therapy (PDT) is a non-invasive therapy for the treatment of cancer as well as many types of non-tumor diseases. Its molecular mechanism is mainly based on three elements: photosensitizers (PS), light of appropriate wavelength and oxygen dissolved in the cells [1], and it has become the fourth most important method of tumor treatment after radiotherapy, chemotherapy and surgery. In clinical practice, PDT has been reported to be used alone and in combination with other therapies[2], such as in the clinical treatment of superficial skin cancer [3], esophageal cancer [4], lung cancer [5] and other oncological diseases and some non-oncological diseases. In addition, a large body of research evidence suggests that PDT has the potential to treat a variety of cancers, such as breast cancer [6], bile duct cancer [7], and pancreatic cancer [7].

1.1. Mechanisms of action of PDT against tumors

The most important mechanism of PDT action on tumors is reactive oxygen species (ROS) killing. After entering the cell, PS absorbs photons and transforms from the ground state to an unstable electron-excited singlet by light irradiation of a certain wavelength, and then progresses to a more stable and longer-lasting electron-excited triplet state by intersystem crossover through the spin-switching of electrons in high-energy orbitals. PDT is usually classified into type I and type II based on the photochemical reaction process of reactive oxygen species. Type I PDT refers to the process in which PS in the excited triplet state reacts directly with triple oxygen ($3O_2$) or biological substrates (such as cell membrane) to produce superoxide anion (O_2^-) and hydroxyl radical ($OH^\cdot$) respectively [8].

However, type II PDT is the process of energy exchange between PS and molecular oxygen to produce highly active singlet oxygen ($^1O_2$) [9]. Type I and type II reactions can occur simultaneously, and the generated ROS can cause oxidative damage to a variety of tumor cells. Although the ratio of the two reactions occurring depends on the substrate, PS, oxygen concentration, and the binding affinity of the photosensitizer to the substrate, in contrast, the type II reaction always dominates during PDT.

In addition, PDT can also achieve tumor killing effect by inducing tumor vascular damage and stimulating anti-tumor immune response.

2. Photosensitizers

2.1. Porphyrins

Porphyrins are essential to the biological activity of all organisms as major components of hemoglobin and myoglobin. It contains 18 π -electron aromatic macrocycles that exhibit characteristic absorption spectra with strong π - π^* leaps in the visible region. Due to its strong $^1O_2$ generation efficiency and excellent fluorescence properties, porphyrin is uniquely suited for PDT.

2.1.1. First-generation photosensitizers. First-generation photosensitizers are mainly mixtures of hematoporphyrin derivatives (HpD). In 1993, Dougherty et al. were the first to extract photosensitizing Photofrin from HpD by purification and chemical modification [10], which is now FDA-approved for the treatment of esophageal and endobronchial cancers in photodynamic therapy, and was the first photosensitizer to receive approval for clinical treatment. Despite the widespread use and efficacy of first-generation photosensitizers in PDT, they still have the drawbacks of low chemical purity and high skin phototoxicity.

2.1.2. Second-generation photosensitizers. Compared with first-generation photosensitizers, second-generation photosensitizers have higher chemical purity, higher yield of ROS formation, better targeting, and better penetration into deep tissues, overcoming certain first-generation photosensitizers' shortcomings [11].

Tookad Palladium-barbiturate (Tookad) is a novel compound derived from the photosynthetic pigment BChl_a, the bacterial equivalent of plant chlorophyll. Using a 763 light source with a light penetration depth of 4 mm, monotherapy with Tookad-based PDT produced a 69% cure rate in subcutaneous tumors within 90 days of treating established bone metastases and orthotopic prostate models. An in situ model of the disease produced similar results [12].

Lebulan 5-aminolevulinic acid (5-ALA) is an intermediate product of porphyrin organism metabolism and can be involved in the biosynthesis of hemoglobin in humans. However, it is not photosensitive and needs to react with an enzyme to produce protoporphyrin IX (PpIX) in vivo before it can play a role.

Npe6 (Laserphyrin, Talaporfin) is a hydrophilic chlorine from chlorophyll a, which can be rapidly cleared from circulation. Npe6 is currently approved in Japan for the treatment of early and advanced lung cancer, esophageal cancer, and intracranial brain tumors [13]. However, there is also evidence that glioblastoma cells (GBM) passing through after the use of Npe6-PDT and short-term relapse may induce a more malignant phenotype in GBM [14].

Foscan tetrahydroxyphenyl chloride (mTHPC, Temoporfin) is a single, pure chlorine derivative with excellent photophysical properties and higher single-site oxygen equivalents [15]. In a study of Foscan-mediated PDT in early-stage oral squamous cell carcinoma (SCC), 85% of protocol-eligible patients achieved a complete tumor response. Patient survival rates were 89% and 75% at one and two years, respectively [16].

Phthalocyanine Phthalocyanine (Pc) exhibits a strong absorption band at 670-770 nm of the spectrum, produces high levels of ROS, and has a long-lived triplet state [17]. The photosensitivity of phthalocyanine can be enhanced when appropriate atoms such as zinc, aluminum, or silicon are present in the center. Wang et al [18] constructed a nanoscale photosensitizer (NanoPcM) by self-assembly of

morphine-substituted silicon phthalocyanine (PcM) and albumin. NanoPcM-based PDT was effective in inhibiting tumor growth, reducing spontaneous lung metastasis.

2.1.3. Third-generation and novel photosensitizers. Due to the poor solubility and low bioavailability of some second-generation photosensitizers in water, the development of third-generation photosensitizers, in which the drug delivery system works in conjunction with second-generation photosensitizers, has become a major research direction [19]. Lu et al [20] developed a composite nanosystem of deep penetration and pH-responsiveness to synergistically enhance the efficacy of combined photothermal and photodynamic therapies against hypoxic tumors. R. Liang and his team [21] constructed a composite nanosystem consisting of polyvinyl pyrrolidone micelles in a carrier consisting of a photosensitizer (zinc phthalocyanine) and a targeting agent (folic acid). They demonstrated that zinc phthalate in the monomeric state contained in the polymer micelles could increase the efficiency of singlet oxygen generation and thus the PDT efficiency.

2.2. Non-porphyrins

The development of nonporphyrin photosensitizers for oncology applications has lagged far behind porphyrin-based PDT. Currently, cationic photosensitizers such as chalcogenopyrylium dyes, benzothiazine derivatives, and a number of thickened cyclic quinones are the main focus of research.

Methylene blue Methylene blue is a thiazine dye widely used in several medical disciplines to produce singlet oxygen and destroy nucleic acids in a nuclease-like manner. Since methylene blue suffers from poor localization to tumors and is easily reduced by cellular enzymes in vivo, nanoparticle-encapsulated methylene blue offers new ideas for methylene blue-mediated PDT in cancer [22].

Hypericin Hypericin is lipophilic and has been shown to be very efficient in generating singlet oxygen and superoxide anions. It has been shown that Hypericin-mediated photodynamic therapy (PDT) can treat nasopharyngeal carcinoma (NPC) by inhibiting matrix metalloproteinase-9 (MMP-9) expression [23].

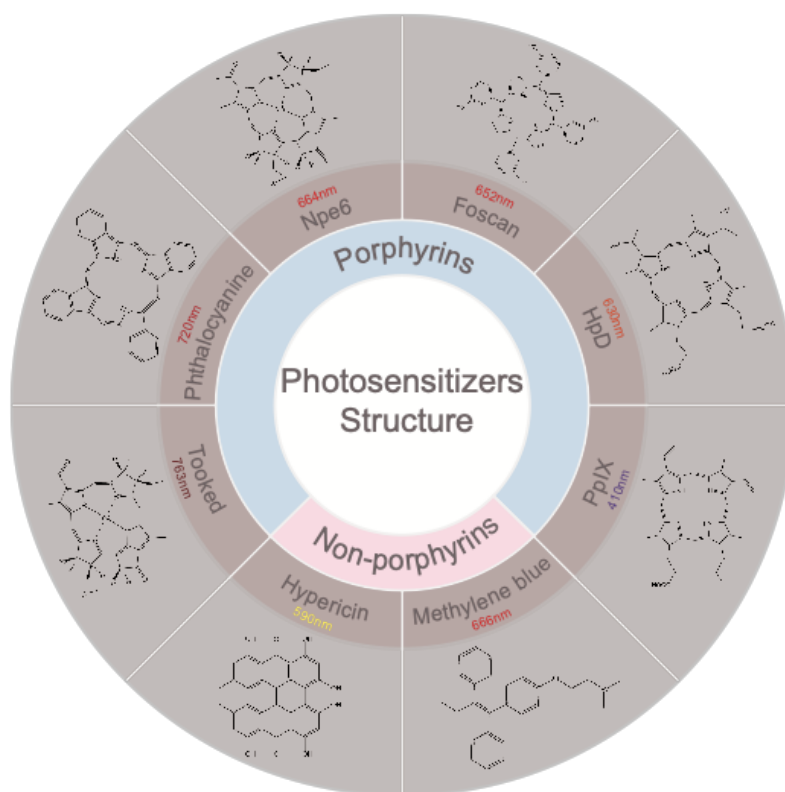


Figure 1. Photosensitizer structures and their therapeutic wavelengths

3. Photodynamic therapy in cancer

3.1. Breast Cancer

Breast cancer occurs in the ductal lining cells of the breast or in the lobules of the breast glandular tissue. Over time, these in situ cancer cells may progress and invade surrounding breast tissue, then spread to nearby lymph nodes or metastasize distantly to other organs in the body. Most patients die from breast cancer due to massive metastasis of the cancer cells. Current treatments for breast cancer usually include surgical excision, radiation therapy and medication.

Wu et al [24] employed nanofactors characterized by high efficiency of ROS generation and low toxicity to treat the 4T1 cell line using 660 nm photoluminescence. After encapsulation, MB-liposomes were observed to have higher in vitro ROS generation capacity than free MB and killed more cancer cells. Machado et al [25] analyzed curcumin (Cur) nanoemulsions as a drug delivery system (DDS+) and used it as a PS in the PDT of MCF-7 cells. The results showed a 10% reduction in the number of live cancer cells and an increase in the number of ROS as seen in the Curcumin is an effective tool for PDT in the treatment of breast cancer.

Triple-negative breast cancer (TNBC), as one of the aggressive breast cancer subtypes, still has no effective treatment to completely cure it. In contrast, Lv et al [26] constructed a multifunctional nanoplateform FA-CD@PP-CpG based on a 4T1 tumor mouse model to synergize with PDT and doxorubicin (DTX) to enhance immunotherapy, which exhibited excellent PDT efficacy and photothermal conversion ability and significantly inhibited tumor growth in vivo under irradiation at 650 nm and 808 nm, respectively. In FA-CD@PP-CpG low-dose loaded DTX could promote the infiltration of cytotoxic T lymphocytes (CTLs), inhibit myeloid-derived suppressor cells (MDSCs), and efficiently polarize them to the M1 phenotype to further enhance the anti-tumor efficacy.

3.2. Lung cancer

Lung cancer is the malignant tumor with the highest mortality rate worldwide and is usually diagnosed at an advanced stage with limited treatment options. Lung cancer is divided into two categories: non-small cell carcinoma (NSCLC) and small cell carcinoma (SCLC).

The common types of NSCLC are squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, which have evolved over the past two decades from the empirical use of cytotoxic drugs to better tolerated and more efficacious regimens by targeting specific molecular subtypes of the disease. Inhibitors of EGFR, ALK, RET, BRAF, ROS1, NTRK, MET, and KRAS are currently used to treat NSCLC harboring the corresponding mutations, but patients almost universally develop resistance during treatment. PDT can restore organ and tissue function in patients with severe ulcers after surgery. In a follow-up case, NSCLC patients were irradiated without tumor treatment at 630 nm for 500 s at 200 J/cm. approximately thirty days after PDT illumination, there were no complications from surgical lobectomy, and there were no signs of disease on imaging and clinical examination 90 days after PDT [27].

3.3. Glioma

Gliomas are tumors that originate from the glial cells of the brain and are the most common primary intracranial tumors. Among them, glioblastoma (GBM) accounts for 57.3% of all gliomas [28] and is the most aggressive glioma [29].

The standard treatment for gliomas is based on surgical resection, combined with a combination of radiotherapy and chemotherapy. However, the unique microenvironment of gliomas, such as the presence of the blood-brain barrier (BBB), oxidative stress, hypoxia, and angiogenesis, make the prognosis of conventional therapies poor. In patients with high-grade gliomas, disease recurrence is almost inevitable. Despite advances in current therapeutic strategies, there are no standard treatment options for recurrent gliomas and recurrent GBM (rGBM).

Over the past 40 years or so, PDT has evolved as a promising therapeutic strategy. In contrast to common PDT tumor-mediated modalities such as uptake, induction of cell death after accumulation of

PS, disruption of tumor vasculature, and enhancement of the tumor immune response, PDT also inhibits the growth and promotes apoptosis of glioma stem cells (GSC). In addition, most of the developed anti-glioma drugs have difficulty in crossing the blood-brain barrier (BBB) in order to reach the tumor site to kill cancer cells, while PDT induces the generation of singlet oxygen ($^1O_2$) leading to an imbalance in endothelial regulation of vasorelaxation resulting in redistribution of calcium, which results in an increase in the permeability of the drug to the blood-brain barrier (BBB) [30].

4. Photodynamic Combination Therapy in Cancer Treatment

4.1. Photodynamic combined immunotherapy

Tumor metastasis has been one of the great challenges in tumor therapy, and promoting the activation of the body's own immune system will help to reduce the probability of metastasis. In PDT therapy, necrotic or apoptotic tumor cells release associated antigens, which induces acute inflammation and APC infiltration in the tumor, further presenting antigens to T cells and activating them to kill the tumor cells. Yu [31] and others constructed nanoparticle GO (HPPH)-PEG-HK as photosensitizers and increased the infiltration of cytotoxic CD8⁺ T-lymphocytes in tumors through activation of dendritic cells, resulting in significant inhibition of primary tumor growth and elevation of antitumor effects in lungs. Gu et al [32], in a retrospective study, evaluated the peripheral blood of 52 patients with stage III-IV colorectal cancer (CRC) who received PDT and the immune cell changes in tumor tissues. The results showed that the local infiltration of various immune cells, such as CD3⁺ T cells and CD4⁺ T cells, was significantly increased in tumor tissues of patients with stage IV CRC, and many inflammatory and immune cells infiltrated into the tumor tissues at 48 h after PDT. The overall survival (OS) of the PDT group was significantly longer than that of the non-PDT group.

4.2. Photodynamic synergistic nanomaterial therapy

Nanoparticle-based PDT is a new therapeutic strategy that relies on nanoparticles as carriers or PS. According to the three key elements of PDT, i.e., PS, excitation light, and oxygen, nanomaterials can target the above to enhance PDT efficacy.

Conventional PS is unstable, untargeted, highly hydrophobic, and susceptible to the body's internal environment, whereas the selection of appropriate nanocarriers is not only effective in improving the biocompatibility of PS, but also in delivering PS to the target cells and reducing the side effects. Li et al [33] designed an efficient nuclear-targeting delivery nanoparticle in which conjugated RGD peptides conferred specific binding and recognition of the nuclear-targeting delivery system to the tumor vasculature and tumor cell membranes to significantly enhance PS specificity and reduce side effects. In addition, nanoparticle loading can facilitate tumor-specific therapy [34].

The excitation light of a single PDT is mainly concentrated in the visible region with limited penetration depth, which does not easily penetrate tumor cells in deeper layers or with chromophores (e.g., melanoma). Indocyanine green (ICG), a multifunctional PS, irradiated at an energy density of 31.2 J/cm², improves the penetration and efficacy of PDT [35]. Automated PDT (APDT), which circumvents the limited penetration depth of conventional PDT, has also been proposed to synergistically enhance the treatment of deep-seated tumors while releasing oxygen [36].

Hypoxia within the tumor is also a major challenge for PDT. In addition to delivering oxygen to the tumor site, elevating peroxidase concentration to catalyze the generation of oxygen in the tumor microenvironment is also a strategy. Tang et al [37] used MnO₂ as a nano-shell to design an H₂O₂-degradable nanoplatform loaded with PS that could react with H₂O₂ and H⁺ in the tumor microenvironment and overcome tumor hypoxia, demonstrating the highest tumor inhibition efficiency. Both in vitro and in vivo studies demonstrated the promotion of oxygen autogenesis for photodynamic therapy.

4.3. Photodynamic synergistic chemotherapy treatment

Combining chemotherapy with PDT has been a major research direction in recent years, as the efficiency of chemotherapy is reduced by severe side effects due to low targeting efficiency and chemoresistance. For the combination of chemotherapy and PDT, some studies have suggested a simple chemical conjugation approach for both agents [38]. However, a key limitation of this strategy is that the drugs do not maintain sufficient bioavailability because they are directly exposed to various enzymes in the bloodstream. Therefore, encapsulation of drugs using nanoparticles is one of the main research directions in conjugated therapy.

He et al [39] used core-shell nanoparticles based on nanoscale coordination polymers (NCPs) carrying a high payload of cisplatin and the photosensitizer pyrolipid, NCP@pyrolipid, for combination chemotherapy and photodynamic therapy (PDT). The results showed superior potency and efficacy compared to monotherapy in low-dose tumor regression (83% reduction in tumor volume) in a cisplatin-resistant human head and neck cancer SQ20B xenograft mouse model. Chu et al [40] developed a pre-drug delivery system that prevents drug leakage during systemic circulation, where the photosensitizer pyropheophorbide-a (Ppa) generated ROS that triggered on-demand release of CPT from local regions and exhibited cytotoxic effects on tumor cells, achieving a comprehensive inhibition of tumor growth.

5. Conclusion

In terms of anti-tumor therapy, PDT, with its unique photoinitiation mechanism and multiple combination therapies, provides more options for patients who cannot repeat surgery or who have seen little success with traditional therapies. In combination therapy, tumor internal hypoxia, PS targeting and cytotoxicity are improved, thus enhancing the anti-tumor effect while maintaining the advantages of PDT. In combination therapy for patients with lung cancer, glioma, breast cancer, etc., PDT kills tumor cells and promotes the cascade reaction of immune cells near the tumor at the same time, promotes drug targeting and drug resistance, and the prognosis of patients with adverse reaction symptoms is not obvious. Currently, clinical evidence for PDT combination therapy still has a long way to go. Since existing research, continued exploration of relevant PS applicable to PDT combination therapy may be expected to prolong the survival time of patients with currently incurable cancers, which has far-reaching significance for the treatment of malignant tumors that need to avoid the risk of surgery as well as to improve the prognosis of patients' quality of life.

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The influencing factors and countermeasures of low fertility in China

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Abstract. Fertility helps maintain the stability of the population and social security to ensure the continuation of economic growth. However, low fertility rate is becoming a problem that plagues the world, which has become particularly serious in China. The relevant research data have shown that the low fertility in China are associate with the changes of physical health status, and survival and social environments. Unless the relevant factors affecting the fertility rate are improved at some stage in the future, these impacts cannot be eliminated in a short time since the low fertility continued over time. Therefore, some measures to improvement of personal physical health, such as forbidden overload work to prompt diseases recovery. In this review, the author explores China's population health issues through analyzing the influence factors for China's declining in fertility rate, aiming to rise people awareness of this important population issue, and provide some possible suggestions to promote population growth by increasing the number of new births.

Keywords: low fertility; physical health; survival environment; social environment

1. Introduction

Fertility is important for maintaining the stability of the population and social security to ensure the continuation of economic growth. However, low fertility rate is becoming a problem that plagues the world, which has become particularly serious in China. In recent years, the relevant research data have shown that the declination of fertility rate in China are associated with the changes in physical health status, and survival and social environments, eventually leading to a series of impacts with the continued fertility declining, such as the pressure on population resources has been effectively alleviated because of more women entering the labor market [1]. In addition, low fertility accelerated population aging, resulted in more pressure on health care, and limited economic growth. Unless the relevant factors affecting the fertility, rate are improved at some stage in the future, these impacts cannot be eliminated in a short time since the low fertility continued over time. Therefore, some measures to improvement of personal physical health, such as forbidden overload work, thereby reducing people's work pressure, will help the recovery of certain diseases that affect fertility. Furthermore, increasing environmental protection efforts will also benefit to establish a good living space that promotes fertility [2]. In response to social factors, formulating more physical in-kind policy and increasing support for kindergarten services will have a positive effect on promoting fertility. In this review, the author explores China's population health issues by analyzing the reasons for China's declining in fertility rate, aiming to rise people awareness of this important population

issue, and provide some possible suggestions to promote population growth by increasing the number of new births.

2. Factors affecting fertility

2.1. Factors affecting fertility in physiology

The living standard has accelerated with the economy developed rapidly after reform and open policy. Many young people have developed bad living habits, including smoking, drinking, overeating, weight loss and excessive dieting, lack of exercise, long-term exposure to electronic products, and staying up late.

In the past few decades, the number of smokers in China has been rising. In addition, as the social economy improves, China's drinking culture becomes increasingly rich, leading to a considerable number of drinkers. The relevant studies have shown that excessive smoking is associated with reduced fertility. No accurate data showed that drinking alcohol is related to fertility. However, the consumption of high alcohol is associated with an increased risk of infertility [3].

Although exact data may be difficult to obtain accurately, the number of people engaged in mental work in China is constantly increasing with long hours work and lack of exercise. Existing research data suggests the exercise duration related to fertility. A survey data obtained from the University of Queensland, showed that the risk of low fertility reduced about 20% in women who have highly active in comparison with low level of physical activity (PA) controls. In addition to this, the level of body mass index (BMI) is negative association with duration of exercise, which is also inversely proportional to fertility [4]. The same survey pointed out BMI is negative relationship with fertility in a dose-response manner. The percentage of low fertility in women with overweight is higher 18% than those with a normal BMI, which reached 36% in obese populations.

The eating habits of Chinese young people have also changed according to economic development. Data shows that the obesity rate among Chinese young people is increasing daily because of much oil and fat in diet. On the contrary, many young people choose excessive dieting to lose weight in order to pursue physical beauty. Indeed, there is a strong correlation between healthy eating habits and high fertility, thereby proper nutrition is beneficial to maintaining high fertility compared to poor eating habits with reduced fertility [5].

Research data shows that radiofrequency or electromagnetic radiation (RF-EMR) derived from wireless devices can damage male reproductive function. High-intensity RF-EMR can also reduce testosterone levels. In addition, RF-EMR have a negative impact on the function of the anterior pituitary gland and the hormones it produces. These influences also become more significant with the increase of RF-EMR intensity and the prolongation of their exposure time [6].

Sleep has been linked to disruption of the menstrual cycle, reduction of sperm motility, and natural fertility. Poor sleep quality includes insufficient sleep, sleep chronotype changing, and night shift work. These behaviors affect reproductive ability through changed the level of melatonin, which acts on the hypothalamic-pituitary-gonadal axis in the endocrine and reproductive systems. For women, poor sleep quality may lead to ovarian dysfunction and menstrual cycle disorders. The later a man goes to bed, the worse his sperm motility. In terms of sleep time types, the nocturnal type has the worst natural fertility compared to the intermediate and morning types. Those sleeping individuals with evening type who prefer to activity in the evening and sleeping late, has the worst natural fertility compared to morning type individuals and intermediate type individuals [7].

2.2. Factors affecting fertility in environment

In addition to personal physiological factors, the impact factors of living environment on fertility cannot be underestimated. Over the past 20 years, public health authorities have focused on identifying environmental chemical substances that can adversely affect hormone function, known as endocrine disruptors (EDs), including chemicals with harmful effects on human's reproductive systems. Studies have shown that endocrine air pollutants can alter fertility by altering the

hypothalamic-pituitary-gonadal axis. Toxic chemicals produced in industry are easily released into the environment and then leach into water or soil, and ultimately enter the human body through the food chain. In addition, air pollutions can also have adverse effects on the fertility of women who use assisted reproductive technology [2].

Through data analysis, the researchers have shown that air pollution has seriously affected the fertility rate of Chinese people, not only having a negative effect on their actual fertility outcomes, but also on their willingness to have children, such as the content of particulate matter (PM)_{2.5} in the air. Moreover, these effects are more significant for couples living in cities with better economic development [2].

In addition to environmental toxicological factors, regional geographical environment also has a certain impact on fertility, such as high-altitude areas like Tibet. Studies have shown that the hypoxic effects of high altitude in Tibet can also impair fertility [8]. The side effects suggest that the fertile population can choose suitable locations to travel for increasing fertility.

2.3. Factors affecting fertility in society

In traditional Confucian concepts of encourage procreation, there is an opposing attitude towards childlessness. But with the development of the ages, people's thoughts have changed. Firstly, public tolerance for childlessness is gradually increasing. Secondly, the group's awareness of independence without children has increased, especially in urban areas. Similarly, the percentage of population who do not rely on their children is increasing compared to twenty years ago [9]. In addition, both the neighbor relationships and local economic levels also affect fertility rates. According to the willingness for second child in women, research data showed significant differences in the factor of neighborhood relationship. In comparison of women who lived in affluent neighborhoods, the women in poorer neighborhoods preferred to having a second child. Communities that provide shared child care facilities increasing women's desire to have children [10].

Social factors that affect fertility also include the education level and income of the public. Among them, the per capita years of education negatively related to the increase in fertility, while the higher education individuals had a positive impact on the fertility rate [1]. The choice of children's number is related to economic factors suggesting that financial support promotes population growth through increasing more children's willing [11].

2.4. Other Factors

Other factors influence fertility include gender, age, and psychological health. In terms of gender, women are more willing to be childless than men. In terms of age, late marriage is positive association with lower fertility rates [9]. Personal psychological stress, such as depression and anxiety, is also an important factor to reduce fertility intentions [12]. The influence factors are list in Table 1.

Table 1. Factors affecting fertility

Classifications	Factors	Examples
Physiological factors	Bad living habits	The risk of low fertility reduced about 20% in women who have highly active in comparison with low level of physical activity controls.
	Wireless devices	
	Lack of sleep	
Environmental factors	Endocrine disruptors	The content of particulate matter (PM) _{2.5} in the air not only has a negative effect on Chinese people's actual fertility outcomes, but also on their willingness to have children.
	Air pollution	
	Regional geography	
Society factors	People's thoughts	The women who lived in poorer neighborhoods preferred to having a second child in compared to affluent neighborhoods individuals.
	Neighbor relationships	
	Local economic level	
	Education level	

3. The influences of low fertility and measures to promote fertility

3.1. *The influences of low fertility*

The decline in the fertility rate means that women are less restricted by themselves, their families or their ideas, which helps more women enter the labor market. In addition, the decline in fertility rate will help alleviate the pressure on consumption of natural resources. However, the decline in fertility also means population aging, which leads to heavier pressure on the medical insurance system and deepening difficulties in social care for the elderly [13]. This trend can be seen from the fact that China's social insurance burden has increased year by year. At the end of 2000, the number of people participating in pension insurance was 136.174 million, which number had reached to 1,053.073 million until 2022. At the same time, the old-age dependency ratio has been increasing from 9.9% in 2000 to 21.8% in 2022 [14].

3.2. *The measures to promote fertility*

Methods to increase the fertility rate in the short term include formulating more in-kind policies and comprehensive support for the three-child policy. Similar to China, South Korea's population has also entered negative growth in 2022. In response to the declining population growth rate year after year, the South Korean government has adopted a series of policies, including providing cash, substances, voucher, and discounts in education and public facilities employing. The research results showed that significant impacts were observed between physical in-kind policy and the initial value of fertility rate [15]. In addition, in view of personal physiological factors, this article advocates that society strengthen the publicity and education of physiological health and fertility, thereby reducing the risk of involuntary infertility. In view of natural environmental factors, the government should increase efforts and at the same time strengthen publicity on environmental protection. In view of social environmental factors, this author recommends increasing support for early childhood services, such as establishing more childcare institutions. In addition to this, attention should also be paid to depression in non-fertile people and providing them with appropriate intervention and treatment.

4. Conclusions

A suitable newborn population can help maintain the stability of population structure and quantity, whereas the reduction of fertility rate has become a challenge in the global public health field. In response to the current downward trend in China's fertility rate, this article analyzes the reasons from three aspects including personal physiology, natural environment, and social environment, introduces the possible related impacts of this trend, and finally proposes a more feasible solution based on the above three aspects. This article draws attention to the phenomenon of declining fertility rates in China.

Although China's declining fertility rate has become a fact, this article believes that the government will further improve relevant policies and take more targeted measures in the future, such as strengthening education and publicity, improving the social support system, and increasing scientific and technological innovation and environmental protection. Since there are international precedents for improving fertility rates through policy measures, such as France, Sweden and other European countries, this article also recommends that China strengthen international cooperation and exchanges, learn from the successful experiences and practices of other countries, and jointly respond to fertility challenges on a global scale, to promote the development of global reproductive health.

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Research progress of photodynamic therapy in antitumor therapies

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Abstract. As one of the leading causes of disease death globally, conventional treatments for cancers have been developed, including surgery, chemotherapy, radiotherapy etc. However, some drawbacks restrain their therapeutic effects. Consequently, researchers have been struggling to develop novel strategies to cure tumors. Photodynamic therapy (PDT) is a significant means that utilizes light as well as photosensitizers to kill tumor cells. In PDT, a photosensitizer absorbs a photon and transfer to states with higher energy, then energy is released and generates free radicals and ground-state molecular oxygen by two distinct types of reaction to exert therapeutic functions. This review concentrates on the antitumor principles and mechanisms of PDT, common photosensitizers, combination of PDT and other cancer therapies and its effects on several typical cancers.

Keywords: Photodynamic therapy, Photosensitizer, Antitumor effect

1. Introduction

Traditional therapies targeting tumor consist surgery, chemotherapy, radiotherapy. Photodynamic therapy act as an adjuvant strategy for diseases, especially for cancers. The principle of photodynamic therapy can be concluded as the photochemical reaction between oxygen, photosensitizer (PS) and light. The process begins when a photon is absorbed by a photosensitizer and the PS is excited to several singlet states with higher energy (S_1, S_2, S_3) [1]. Then by the emission of energy as fluorescence or heat, the PS can go back to the ground state. Additionally, intersystem crossing after a spin inversion could produce photosensitizer in an excited triplet state T_1 which has a longer living period. The T_1 state is a significant feature for two kinds of photochemical reactions that possess therapeutic effects, producing free radicals (Type I reaction) and ground-state molecular oxygen (Type II reaction) respectively [2]. The photodynamic therapy tolerance for patients is optimistic due to its selectivity, provided by illumination volume control of certain tissues and localization of photosensitizers and chromophores to targeted tissues [3].

2. Antitumor Mechanisms of PDT

2.1. ROS damage

Most of the application of PDT take advantage of type II reaction, generating singlet oxygen $^1O_2$ which exerts primary cytotoxicity on malignant tumors. Its reaction with lipids on the membrane leads to lipid

peroxidation and damage of cellular membranes. Besides, its reaction with amino acid affects functions of proteins [4]. However, the excessive proliferation and lack of blood supply form a hypoxic tumor microenvironment restricts the generation of ROS. Therefore, attempting to change the low-oxygen condition is of vital importance and many methods have been organized to alleviate hypoxia and promote the generation and tumor lethality of ROS [5].

One of the common strategies is to decompose H_2O_2 to generate O_2 . Chen et al. developed a novel pH/ H_2O_2 responsive HSA-MnO₂-Ce6&Pt (HMCP) nanoparticle was generated by a biomineralization process based on albumin. MnO₂ nanoclusters involved in the system would react with endogenous H_2O_2 inside tumor microenvironment (TME) when it was injected into the tumor issue, producing oxygen, relieving hypoxia and enhancing PDT cancer treatment [6].

Another method is to deliver exogenous oxygen directly. It is well known that red blood cells (RBC) take the responsibility to deliver oxygen in body. Zhang et al. constructed a “NanoRBCs” by assembling nanoliposomes based on PFOB (perfluorooctyl bromide), PFC-based blood substitution. A heptamethine dye IR780 was also added into the structure to enhance PDT efficacy. The PFOB@LIP-IR780 “Nano-RBCs” represented a high oxygen-delivery feature with enlarged PDT and mitochondria-targeting characteristic which could basically eliminate tumors. [7].

Other treatments to reduce hypoxia include normalization of tumor blood vessels, perfluorocarbon (PFC)-based and O_2 carriers based on metal-organic framework (MOF), reducing tumor oxygen consumption, destructing the extracellular matrix of tumor and inhibiting HIF-1 signal pathway [8].

2.2. Necrosis and Apoptosis

Cell apoptosis and necrosis can be induced by Ca^{2+} release from the endoplasmic reticulum (ER), regarded as Ca^{2+} -regulated mitochondrial cell death. To further investigate the variations of the level of Ca^{2+} in tumor after the PDT, Zhao et al. utilized Ca^{2+} fluorescent indicator Rhod-3 AM to determine Ca^{2+} concentrations intracellularly. Together with elevating drug concentration, the A549 cells showed an increase of Ca^{2+} level after PDT treatments. The ER stress caused by photosensitizer EB-ER-Pc (EB: erlotinib; ER: ER-targetable moiety methyl sulfonamide; Pc: photosensitizing core) could rise calcium ion standard by causing ER stress. Therefore, induction of apoptosis and necrosis were achieved by the generation of ROS and excessive Ca^{2+} [9].

There are two major pathways considering apoptosis, extrinsic/death receptor-mediated apoptosis and intrinsic/mitochondria-mediated apoptosis. The death receptor-mediated pathway occurs when death ligands, such as FasL, TNF- α , TRAIL, attach to death receptors (DR) on cell surface, including Fas, TNF-RI, TRAIL receptor, forming the death-inducing signaling complex (DISC). It serves as a platform to recruit the initiator procaspases-8 as well as initiator procaspases-10 and lead to their activation, cleaving and activating procaspases-3 and initiator procaspases-7 to achieve apoptosis [10]. As for mitochondria-mediated pathway, an example is a pH-activatable nanoparticle M-TPPa designed by Qi et al. which could accurately target both early endosome and mitochondria and amplify theranostic signal for effective therapeutic results. M-TPPa could be rapidly absorbed by tumor cells by endocytosis in the acidic environment, accumulated into the mitochondria and activated the fluorescence signals and photoactivity of photosensitizer, inducing dysfunction of mitochondria and intrinsic tumor cell apoptosis [11].

2.3. Autophagy

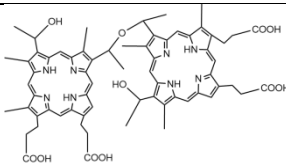
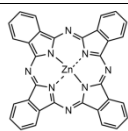
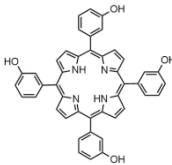
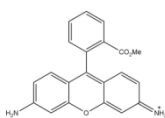
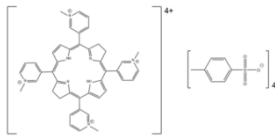
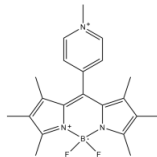
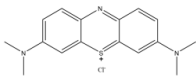
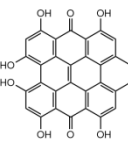
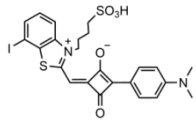
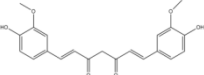
Autophagy can be regarded as a double-edged sword which contains both pro-survival mechanism via the clearance of destroyed cellular components and a death mechanism, which particularly enhance organelle destructions. A vesicle with bilayer film called autophagosomes engulfs impaired materials and isolates them from the cytoplasm. After they are fused with lysosomes, autolysosome, a single-membraned vesicles, is formed to degrade autophagic cargos by lysosomal hydrolases [12]. The degradation contributes to the deletion of unfolded proteins, destructed organelles, microorganisms and recycling of cellular components and nutrition to tumor issues [13].

Function of autophagy relies on the damage standard of PDT. Once autophagy is activated, cell death can directly happen as a result of autophagy-dependent cell death. Considering the AKT-mTOR regulation system, 450-nm blue laser (BL) with PDT mediated by sinoporphyrin sodium (DVDMS) to treat human gastric cancer (GC) is researched by Li et al. The results showed apparent inhibition of cell growth and induction of apoptosis in GC in vitro by the generation of excessive ROS when treated with 450-nm laser-mediated DVDMS-based PDT. Additionally, autophagy flux was induced by BL-PDT in GC cells and the induction of autophagy was illustrated to offer positive contribution to apoptosis induced by BL-PDT on GC cells. Taking advantage of analyzing methods such as transcriptomic analysis, ROS/PI3K/AKT/mTOR pathway was proved to take part in the control of autophagy [15].

3. Photosensitizer

From the mechanism of photodynamic therapy, it is apparent that photosensitizer is of vital importance in PDT. Generally, PSs can be categorized into three generations. Some typical photosensitizers are listed in the Table 1.

Table 1. Typical photosensitizers.

Category	Name	Structure	Category	Name	Structure
Porphyrin	Photofrin		Phthalocyanine	Liposomal ZnPC	
Chlorin	Foscan		Rhodamine	Rh123	
Bacteriochlorin	Tetrahydroporphyrin-Tetratosylat (THPTS)		BODIPY	DIMPy-BODIPY	
Phenothiazinium salt	Methylene Blue		Perylenequinone	Hypericin	
Squaraine	ASQI		Curcuminoid	Curcumin	

3.1. First-generation Photosensitizer

The first discovered PS agent was porphyrin excreted in urine, also known as uroporphyrin. However, it was not put into practice for PDT in the beginning [16]. From 1950s to 1960s, Schwartz and Lipson and their colleagues figured out a way to develop a sensitizer with a refined form that is more active called Hematoporphyrin Derivative (HpD), which was the first PS that was applied in clinical uses to treat bladder cancer and commercialized as Photofrin® by Dr. Thomas Dougherty, a mixture containing dimers and oligomers that are water soluble but lipophilic with 2 to 9 porphyrin units connected by ether bonds [17]. Wang et al. discovered that in Extramammary Paget's Disease (EMPD), HpD-PDT resulted

in relatively high complete remission (CR) rate. However, in this study, pain during light irradiation was considered to be the major unwanted result and there are plenty of distinct side effects including partial irritation, photosensitivity, scar generation, infection of urinary tract, hepatic injury and uroschisis in the previous researches. Moreover, considering esophageal carcinoma, the primary disadvantage of HpD-PDT was the extended photosensitivity of the skin [18]. Therefore, photosensitizers that possess less adverse reactions still required to be developed.

3.2. Second-generation Photosensitizer

The second-generation photosensitizers include chlorin, benzoporphyrin derivatives, texaphyrins, bacteriochlorin analogues and phthalocyanines. Common properties of second-generation PSs include higher chemical purity, higher singlet oxygen production and better penetration capability. Additionally, they illustrate fewer side effects as they own better selectivity for tumor sites and faster eradication speed. However, the major drawback of the second-generation PS is their poor water solubility, limiting intravenous administration [19].

Meso-tetrahydroxyphenylchlorin (mTHPC), also known as Foscan®, is a clinically approved chlorin photosensitizer. Haimov-Talmoud et al. designed a Ce-doped- γ -Fe₂O₃ maghemite nanoparticles (MNPs) combined with mTHPC, which showed high uptake and death rate in cancer cells after PDT in MDA-MB231 (human breast cancer) cells. What's more, PDT results in a decreased tumor size and significant tumor regression [20]. A novel bacteriochlorin-based photosensitizer FBC was developed by Wu et al., which could not only generate oxygen-dependent ¹O₂, but also produce oxygen-independent ROS O₂^{•-} and OH[•], relieving tumor hypoxic microenvironment and enhancing therapeutic effects [21].

3.3. Third-generation Photosensitizer

The third generation aims at optimizing delivery process and targeting tumor cells. To promote the selectivity, several measurements have been taken by scientists, including combined second-generation PSs and molecules that targets certain receptor, assemblies of PSs with low density lipoprotein, conjugation of PSs with antibody that targets certain antigen, the utilization of markers for tumor surface including growth factor receptors, transferrin receptors or hormones [19].

4. PDT Combined with Other Cancer Therapies

4.1. PDT Combined with Immunotherapy

Traditional strategies against cancer include surgery, radiotherapy, chemotherapy, endocrine therapy and biological therapy, which have limitations such as high aggressiveness, undesired therapeutic effect, toxicity, tumor specificity deficiency and the possibility of recurrence of tumor. Therefore, novel treatments have been applied in cancers and immunotherapy is one of them developed to promote natural defenses to eliminate malignant cells [22].

However, complicated tumor immunosuppressive microenvironment restrains the therapeutic effect of immunotherapy, which can be greatly improved with the aid of phototherapy. Wang et al. focused on the membrane-targeted photosensitizer TBD-3C that was able to induce immunotherapy efficacy containing aggregation-induced emission (AIE) property via photodynamic therapy. Not only did pyroptotic cell serves as a tumor-associated antigens (TAAs), secreting cell contents (e.g. cytokines and proinflammatory factors), but also it emitted signals of danger-associated molecular patterns (DAMPs), indispensable for the recruitment and maturation of antigen-presenting cells (APCs). Induced by TBD-3C, it stimulated M1-polarization of macrophages, the ripeness of dendritic cells (DCs), and upregulation of CD8⁺ cytotoxic T-lymphocytes (CTLs), transforming immunosuppressive “cold” TME to immunogenic “hot” TME and inhibiting the growth of pancreatic cancer [23].

4.2. PDT Combined with Chemotherapy

Chemotherapy is a conventional cancer therapy that utilize cytotoxic drugs to attack rapid proliferating cells, widely regarded as the first option of tumor metastases. However, shortcoming of it is that it also

influences normal cells that split rapidly including cells in digestive tract and the bone marrow [24]. Consequently, combination strategies arouse more and more attention aiming at achieving synergistic effect and reducing side effects at the same time and nanotechnology is widely applied in recent studies.

In the research of Su et al., a polymer PEG(-b-PCLCe6)-b-PBEMA with star shape was designed to improve ROS production of photodynamic therapy and the reduction of GSH in situ in tumor cells. The conjugation of polycaprolactone (PCL) and photosensitizer Ce6 composed lipophilic part to assist the formation of micelle and fulfilled photodynamic therapy under illumination. The H₂O₂-labile group of arylboronic esters played a character of producing H₂O₂-induced quinone methide (QM) to deplete GSH. Besides, this artificial micelle could load Doxorubicin (DOX) and carry DOX into tumor tissue in high efficiency as well, resulting in the fusion of photodynamic therapy and chemotherapy [25].

4.3. PDT Combined with Photothermal Therapy (PTT)

PTT is a treating method based on photothermal conversion, which means PTT agents generate heat when they are exposed to light [26]. Electrons of ground state transfer to excited state when light is absorbed by the agents, generating electronic excitation energy that could cause overheating of topical environment. The produced heat has the effect of aggregation and denaturation of proteins, evaporation of cytosols and lysis of cells. However, PTT also has many drawbacks such as limitation of penetration depth for NIR light, photobleaching leads to a reduction in therapeutic effect, high risks of cancer relapse and metastasis. Additionally, incorrect dosage may result in tissue burning, swelling, and inflammation [27].

To combine PDT and PTT, Li et al. selected BD3, 1,10-phenanthroline-2,9-dimethyl-linked berberine dimer, from all of four compounds BD1, BD2, BD3 and BD4 (BD1 and BD2: pyridine-2,6-dimethyl-/2,2'-bipyridine-3,3'-dimethyl-tethered berberine dimers, BD4: tetrakis(4-benzyl)ethylene linked berberine tetramer), which possessed the lowest singlet-triplet energy level difference (ΔE_{ST}) and most generation of ¹O₂, to build AuNSs-BD3@HA (abbreviation ABH, AuNSs: gold nanostars; HA: hyaluronic acid). Combined PDT and PTT anti-breast cancer therapeutic effects were achieved by BD3 and AuNSs respectively, together with the targeting-CD44 (a variety of receptor overexpressed in cancer cells) property of HA [28].

5. The Antitumor Effects of PDT

5.1. Breast Cancer

As the most familiar malignancy in female, breast cancer (BC) usually generates in the breast lobules, tubes, or connective tissue [29]. In spite of improvements of mammographic breast screening, surgery, chemotherapy and the use of adjuvant hormonal therapies have been increasing the survival rate of breast cancer patients, novel technologies are still in need to for patients who are not eligible or responsive to standard managements [30]. Therefore, a rising number of scientists have been investigating new PDT-assisted immunotherapy nanomedicines for breast cancer [31].

To obtain clear images of magnetic resonance imaging (MRI) for cancer diagnosis using transition metal manganese ion (Mn²⁺) and treating tumor by PDT, Zhou et al. created a dendrimer composite-based self-assembly nanoparticle by combining hypericin, MnO₂ particles and polyglycerol dendrimer with cysteine (G1-Cys). In this research, not only did Mn²⁺ show well T₁-weighted imaging (T₁WI) performance, it could also convert H₂O₂ into hydroxyl radicals, intensifying PDT therapeutic efficacy. Moreover, activation of the photosensitizer hypericin resulted in the production of ROS, thus amplifying the treatment effect [32].

5.2. Lung Cancer

Lung cancer is the uppermost reason of cancer death in the world, killing more people than colon, breast, and prostate cancers together and is the major cancer death cause in men and the second highest cancer death cause in women, just behind breast cancer [33]. It can be categorized into small-cell carcinoma (SCLC, takes up 13%) and non-small-cell carcinoma (NSCLC, takes up 83%) [34]. Even though PDT

has limitations such as short half-life in plasma, insufficient tumor tissue infiltration and moderate specificity, it is extensively studied in lung cancers to reduce adverse effects caused by conventional therapeutic strategies due to its ability to reduce drug resistance and minimize toxicity.

Epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) is a drug targeting NSCLC which resistance has become a major hindrance to treat NSCLC and Yes-associated protein (YAP) regulates resistance to EGFR-TKI. Huang et al. created a stimuli-responsive and block dendritic polymer to load EGFR-TKI gefitinib (Gef), YAP-siRNA and photosensitizer polymer-pyrropheophorbide a (Ppa) and irradiate light to eliminate EGFR-TKI resistant cancers. Apart from the inhibition effect of Gef targeting EGFR signaling, YAP-siRNA could also block the MAPK/ERK and PI3K/AKT pathway to inhibit tumor cell proliferation and Ppa-based PDT induced tumor apoptosis by generated ROS [35].

5.3. Glioma

Glioma is one of the most common central nervous system (CNS) primary malignant tumor, taking up about 30% of primary brain and CNS tumors and 80% of brain cancers [36]. With special features like hypoxia, the blood-brain barrier (BBB), ROS and angiogenesis, the treatment efficacies of conventional therapies are restrained. In most cases, anti-glioma drugs have troubles in crossing BBB to get to tumor sites. Furthermore, promotion of tumor initiation and progression are also achieved by oxidative stress and hypoxia-induced invasion of glioma, drug resistance, and angiogenesis contributes to the growth of cancer and leads to the tumor recurrence [37].

The internalizing RGD peptide (iRGD), a cyclic peptide with 9 amino acids, includes a tumor cell targeting motif CendR. Lu et al. produced a prodrug polymer that contained chemotherapeutics camptothecin (CPT), polyethylene glycol (PEG), modified iRGD peptide with disulfide bond conjugation, forming CPT-S-S-PEG-COOH nanosized polymeric micelles with photosensitizer IR780. The modification of iRGD allowed micelles to pass through the BBB and target glioma cells via ligand transportation mediated by $\alpha_v \beta$ integrin and neuropilin-1 both in vitro and in vivo, possessing a more excellent tumor-eliminating efficacy [38].

6. Conclusions

The research progress of photodynamic therapy shows that it is an effective way to treat cancers mainly by ROS damage, necrosis, apoptosis and autophagy mechanisms. In addition, the combination of PDT with other treatments for cancers enhances the therapeutic efficiency and PDT is proved to be effective in various cancers. However, the major challenges of PDT are the hypoxia TME, limited depth of light penetration and the selectivity of PSs, which restrict the application of photodynamic therapy. As a result, numerous attempts have been made to solve these problems in the past few years. The rapid development of nanocarriers for PSs delivery makes it possible to elevate PDT efficiency and selectivity. With the further development of nanotechnology, photodynamic therapy possesses a fascinating opportunity to play an indispensable character in cancer treatments and widely applied in clinical practice.

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Research progress of curcumin and its nanodelivery system in anti-tumor field

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Abstract. The focus of attention these days is on cancer prevention and treatment, as it is the second leading cause of death worldwide and its incidence continues to rise year. Due to its interactions with different cells and molecules, curcumin, a polyphenol natural substance derived from the turmeric plant, has numerous important applications in malignant disorders. Curcumin has demonstrated a high usage value and important benefits in the treatment of tumors; however, it has certain drawbacks, including low solubility, poor targeting, fast metabolism, and poor stability. The goal of this article is to enhance curcumin's bioavailability and enhance the therapeutic impact of various tumor illnesses by means of a cooperative nano-drug delivery system involving chemotherapeutic medicines and curcumin.

Keywords: curcumin, nano delivery system, anti-tumor therapy

1. Introduction

As the world's greatest cause of mortality, cancer poses a serious threat to public health [1]. Statistics show that in 2022, there were around 20 million new instances of cancer and 9.7 million cancer-related fatalities [2]. Traditional Chinese Medicine-combination therapy is a new treatment approach and an important cancer treatment strategy as it shows promising anti-tumor potential [3]. Curcumin is a natural polyphenol component from turmeric root, the main bioactive component of turmeric spice, in the diketone group [4]. Curcumin has a wide range of pharmacological activities, such as antimicrobial, anti-tumor, anti-inflammatory, antiviral, and antifungal qualities. It also plays a variety of roles in regulating enzymes, adhesion molecules, transcription factors, protein kinases, cytokines, and REDOX balance involved in inflammation [5].

In recent years, curcumin in the field of anti-tumor research has been more and more in-depth, according to the horizontal molecular experiments related studies show that curcumin's main anti-tumor mechanism is to increase the target of tumor cells to drugs, regulate the transduction of proliferation and apoptosis signaling pathways and the expression of related enzymes, proteins and genes. Curcumin has a lot of potential, however its bioavailability is limited due to its low water solubility and quick bodily degradation. Numerous nanoformulations have been developed as a result of recent advancements in nanotechnology to solve these pharmacokinetic restrictions. [6], such as nanotechnology-based systems, liposome encapsulation designed to enhance their absorption and efficacy [7].

2. Curcumin nanodelivery systems

2.1. Nanoparticle

Lipid nanoparticles, which are nanoparticles constructed using phospholipids or phospholipid derivatives [8], are the basic building blocks of nanotechnology and vary in size between 1 and 100 nanometers [9]. Many organic nanoparticles have been designed as curcumin delivery vehicles as anti-cancer therapy alternatives because of the advantages of nanoparticle administration, including biocompatibility, decreased toxicity, enhanced permeability and retention (EPR) effects, and precision targeting [10]. To improve the drug loading capacity, Guo et al. designed a REDOX-responsive lipid prodrug C210 nanoparticle delivery system to form nanoparticles with high drug loading capacity, which can improve the bioavailability and anti-tumor activity of curcumin derivative C210 [11]. Liu et al. prepared the early irinotecan hydrochloride-curcumin nanosystem (SICN). SICN is a promising carrier-free nanoparticle. When exposed to the acidic environment of tumors, SICN showed stronger cytotoxicity due to charge conversion, opening new ideas for combination therapy of tumors [12]. Li et al. designed a core crosslinked nanoparticle (CCL NPs). The results show that CCL NPs with surface-modified antibodies can actively target tumor cells, and the tumor inhibition rate is as high as 78.7% in vivo experiments. This work provides new ideas for the design of nanomaterials targeting breast cancer [13].

2.2. Liposomes

The liposome is a double-layer vesicle composed of phospholipids [14], with an aqueous core inside, which can encapsulate up to two therapeutic agents, and has high stability and efficient drug loading capacity [15]. At the same time, the liposome can be functionally modified to make it have specific recognition functions such as targeting [16]. Alharbi et al. designed exosomes and liposomal nanocarriers of mangiferin and curcumin, targeting the PI3K/Akt/mTOR pathway, protecting the compounds from degradation, allowing controlled release and improving cellular uptake to enhance drug delivery [17]. Liu et al. prepared FC-DP-LIP containing curcumin and 5-fluorouracil by thin film water method. It had good morphology, stability and drug encapsulation rate, showed good biocompatibility and was cytotoxic to a variety of cancer cells [18]. Li et al prepared novel liposomes (CUR-APR/HA&GA-LPs) modified with hyaluronic acid (HA) and glycyrrhizin (GA) for co-delivery of Aprepitin (APR) and curcumin (CUR) and induction of tumor cell apoptosis using CUR. The results showed that CUR-APR/HA and GA-LPs can inhibit tumor proliferation and metastasis, reduce extracellular matrix (ECM) deposition and tumor angiogenesis, and have a good anti-liver cancer effect [19].

2.3. Polymer micelles

Micelles are formed by self-assembly of an amphiphilic polymer at a critical micellar concentration (CMC), with a hydrophobic tail and a hydrophilic head [20]. Micelles not only increase the solubility of hydrophobic drugs, but also increase their biocompatibility, prolong the circulation in vivo and prevent the rapid breakdown of drugs [21], showing good prospects in tumor therapy [22]. Zhou et al. prepared multi-pH-sensitive polymer-drug conjugate mixed micelles, which can effectively co-deliver doxorubicin and curcumin, which can greatly reduce the side effects of doxorubicin, and have synergistic enhanced anti-tumor and anti-metastasis effects [23]. Liu et al. synthesized a novel REDOX-responsive mPEG-SS-PLA (PSP) polymeric micelle for the delivery of sorafenib (SAF) and curcumin (CUR). The study showed that mPEG-SS-PLA polymer micelles loaded with SAF and CUR showed enhanced therapeutic effects on hepatocellular carcinoma in both in vitro and in vivo models and had broad application prospects in cancer therapy [24].

3. The application of curcumin in the field of anti-tumor

3.1. Breast Cancer

Breast cancer (BC) has become the fifth most common cause of cancer-related morbidity. Worldwide, breast cancer accounts for approximately 30% of cancers in women, with a mortality to morbidity ratio of 15% [25]. The researchers found that BC can be fought with a curcumin nanodelivery system [26]. Lv et al. synthesized a curcumin-carrying polymer nanocarrier (HA-b-PCDA). Molecular docking and molecular dynamics studies showed that these drug-carrying HA-b-PCDA nanoparticles significantly inhibited the proliferation and stem cell formation of BCSC-rich 4T1 mammary spheres, and could deliver a variety of clinical chemotherapy drugs [27]. Novel core-shell nanoparticles with controlled release properties for zinc ions and curcumin were successfully created by Gao et al. The amounts of ZIF-8, CUR, SF, and PDA in a hybrid nanoplatfrom were carefully controlled for the first time using microfluidic fast mixing to enhance anti-cancer efficacy [28].

3.2. Lung Cancer

Lung cancer is the most common cancer globally in terms of diagnoses [29], accounting for 1.79 million deaths and 2.2 million new cases yearly, making it the leading cause of cancer-related death globally [30]. Radiation therapy, chemotherapy, targeted therapy, and surgical excision are the current therapeutic approaches. Even though other EGFR tyrosine kinase inhibitors (EGFR-Tkis) have been developed, such as erlotinib (Er), acquired resistance to these medications presents a major challenge and lowers their long-term effectiveness in treating lung cancer [31]. As a result, curcumin nanodelivery technologies present an innovative therapeutic strategy. Chen et al. combined Er with curcumin (Cur), an important ingredient in traditional Chinese medicine, to improve the treatment's effect on lung cancer. By preserving the level of ikappa-B expression and suppressing the production of PI3K in the EGFR downstream signaling pathway, Cur can prevent Er resistance by preventing the growth of tumors. Sasikarn Sripetthong developed curcumin-loaded nanomicelles using quaternium-salt chitosan and vanillin imide (QCS-Vani) conjugates as carriers and investigated their entrapment rate. According to the study, curcumin-loaded QCS-Vani nanomicelles triggered apoptosis in lung cancer cells and shown better cytotoxicity and selectivity against the lung cancer A549 cell line and decreased toxicity against normal cells (H9C2) [32].

3.3. Liver Cancer

Liver cancer, particularly hepatocellular carcinoma (HCC), is a major global health problem due to its high prevalence in many countries [33]. The high mortality rate of HCC should be attributed to late diagnosis and limited treatment. Drug therapy is a common palliative strategy for clinical treatment. However, they have many disadvantages such as low selectivity, dose restriction, toxicity, and adverse side effects [34]. Therefore, identifying new, effective codosing system dosage forms remain an important research focus. The use of lipid nanoparticles as delivery vehicles for insoluble medications like curcumin has been investigated. According to Wei et al., curcumin-human serum albumin nanoparticles modified with a peptide that binds to programmed death ligand 1 (PDL1) can enhance the delivery of drugs targeted at tumors while also reestablishing the immune system by preventing the PDL1/PD1 connection and coordinating anti-cancer efforts. [35]. Curcumin/triphenylphosphine bromide chitosan-G-poly (TPP-CZL) nanomicelles have been shown by Li et al. to significantly reduce mitochondrial membrane potential, increase the expression of pro-apoptotic protein Bcl-2, decrease the expression of anti-apoptotic protein Bax, and promote apoptosis in liver cancer cells based on cell survival rate and Hoechst staining results [36].

4. Summary

Because they are safe and effective, researchers are considering the use of natural compounds as potential chemotherapeutic medicines. The active hydrophobic polyphenol curcumin, which is derived from the rhizome of the turmeric plant, has demonstrated promise as a cancer treatment. We have been

obliged to refocus our attention from a single target to the regulation of the body's network due to the substantial obstacles presented by cancer, as well as the bad responses and drug resistance brought on by prolonged therapy with a single agent. The emergence of nanobiotechnologies has further opened vast opportunities to explore and expand the use of curcumin in the medical field. Nanodelivery systems using curcumin and its derivatives have better bioavailability and solubility, have clear advantages in anti-tumor therapy, and can deliver drugs to different targets or sites of action. As reported in this review, the curcumin nanodelivery system has attracted much attention, which will become a new method of cancer treatment, and it is believed that curcumin will have more extensive and important applications in the anti-tumor field in the future.

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Research advancements on the correlation between sarcopenia and gastroesophageal reflux disease

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Abstract. With the advancement of population aging, it is increasingly vital to focus on the health conditions of the elderly. Sarcopenia is a condition characterized by gradual and systemic loss of skeletal muscle that may result in various outcomes, such as vulnerability, injuries and metabolic disorders, its impact on the lives of the elderly can't be ignored. Non-erosive reflux disease (NERD) is the most prevalent phenotype of gastroesophageal reflux disease (GERD), a major clinical gastrointestinal illness. Therefore, this article provides an overview of the definition, diagnosis, epidemiology, pathophysiology, and treatment of GERD and sarcopenia, with the intention of offering fresh viewpoints to improve the elderly's quality of life.

Keywords: gastroesophageal reflux disease, sarcopenia, relationship

1. Introduction

Sarcopenia, as a chronic disease that leads to muscle loss and decreased strength [1, 2], is associated with mobility limitations, reducing the quality of life [3], and complications such as metabolic syndrome [4]; prevalence of GERD increases over time in the elderly [5]. With the aging of society and growth of population, it will increase the public health burden [6]. Therefore, this article provides an overview of the definition, diagnosis, epidemiology, pathophysiology, and treatment of GERD and sarcopenia, with the intention of offering fresh viewpoints to improve the elderly's quality of life.

2. The definition and diagnosis of GERD

The lower esophageal sphincter temporarily relaxes in GERD patients, leading to weakening of the anti-reflux barrier and its main clinical manifestations are heartburn and reflux [7]. Research has demonstrated a correlation between GERD incidence and age [8]. Thus, public health burden increases as society aging and the population grows [6]. The diagnosis of GERD lacks an absolute standard. Therefore, symptoms like reflux and heartburn are used to make the diagnosis. Esophageal mucosal endoscopy, dynamic reflux monitoring can be used to evaluate esophageal acid exposure and high-resolution manometry can be used to assess motility disorders associated with GERD [9]. The Lyon Consensus 2.0 [10] enhances diagnostic specificity and offers a contemporary diagnosis for GERD: for undiagnosed GERD patients, esophagogastroduodenoscopy and/or outpatient reflux monitoring can provide conclusive evidence; ancillary examinations like endoscopy, pH impedance monitoring, and manometry may impact the conclusion when the evidence is inconclusive; the only approach for

determining whether a patient with GERD is experiencing refractory reflux is through pH impedance monitoring.

3. The definition and diagnosis of sarcopenia

Sarcopenia is characterized by a progressive loss of both skeletal muscle quality and strength. in skeletal muscle quality and strength [11], primarily due to long-term accumulation of adverse changes in the muscles. A meta-analysis by Petermann and colleagues [12] indicates that the incidence of sarcopenia under the diagnostic standard of the European Working Group on Sarcopenia in Older People 2(EWGSOP2) is 10% (95% CI: 0.02-0.17). Sarcopenia has several diagnostic criteria. Currently the most commonly used diagnostic standard of sarcopenia has been revised by EWGSOP2 for the algorithms used in identifying and diagnosing of sarcopenia [13]: (1) The amount of muscle mass in the limbs, for males should be less than 20 kilograms, and for females less than 15 kilograms; (2) Low muscular strength is defined as grip force below the normal range, with less than 27 kilograms for males and less than 16 kilograms for females; (3) a step rate of ≤ 0.8 m/s, a score of ≤ 8 on the Simple Physical Assessment Scale (SPAS), and a chair stand test of 5 repetitions >15 s. Reduction of muscular mass, combined with a decline in muscle strength or low fitness, can be used to diagnose sarcopenia.

4. The epidemiology of GERD and sarcopenia

4.1. Researches on the risk of developing sarcopenia among patients with GERD

A cross-sectional study by Kim and his colleagues [14] which included 8,218 patients (3,414 in the GERD category and 4,804 in the non-GERD category) found that 16.8% of GERD patients suffered from sarcopenia and sarcopenia is an independent predictor of GERD. Besides sarcopenia incidence was significantly more in the GERD category compared to the non-GERD category. Imagama and colleagues [15] conducted a prospective longitudinally cohort investigation with a 5-year follow-up and discovered that sarcopenia and reduced back muscle strength were important risk factors for the onset of GERD.

4.2. Researches on the relationship between GERD and sarcopenia

There is a current study [14] indicating that GERD is connected to sarcopenia. Sarcopenia may cause metabolic syndrome [4] which affects the mechanical barrier's function of the esophagus and stomach, so this triggers gastroesophageal reflux. Meanwhile cytokine signaling may play a role between sarcopenia and GERD [16-18]. A Mendelian study by Hu and colleagues [19] also discovered that features associated with sarcopenia such as decreased grip strength, decreased walking speed, and decreased appendicular lean mass were causally related to GERD. There is a deficit of sufficient evidence to substantiate a direct link between GERD and sarcopenia, so further analysis of the pathophysiological mechanisms of GERD and sarcopenia is needed.

5. Researches on the shared pathophysiological mechanisms of GERD and sarcopenia

5.1. Inflammations

Inflammation is one of the most crucial pathophysiologic mechanisms of gastroesophageal reflux. Gastroesophageal reflux overactivation of NF- κ B increases cytokine levels such as TNF- α and IL-6 in esophageal epithelium [16]. One of the most important pathophysiologic mechanisms of sarcopenia is also inflammation. In a multicenter prospective longitudinal study by Li and colleagues [17], sarcopenia patients had significantly higher inflammatory mediator (IL-6, IL-18, TNF- α) levels in serum compared to non-sarcopenia patients. In the meantime, a study found that lower skeletal muscular strength and lower quantity of muscle were substantially correlated with greater levels of circulating inflammatory markers, with IL-6 and TNF- α being particularly linked to these outcomes [18]. Systemic inflammatory indicators typically rise with age, with TNF- α being one of the main culprits [20]. Elevated TNF- α levels (>11.15 pg/ml) have been linked to a 7.6-fold increase in the likelihood of sarcopenia [17]. By activating the ubiquitin-proteasome pathway, TNF- α causes a disorder in the production and metabolism of

proteins in skeletal muscle, which results in atrophy and a loss in skeletal muscle mass [21], thus, both muscle mass and strength in skeletal muscles are reduced [18].

5.2. *Body mass index and obesity*

A cohort study showed higher BMI independently linked to GERD symptoms [22]. It has also been shown that there is a genetic link to the greater BMI susceptibility, which increases the chance of having GERD [23]. Obese subjects have an increased prevalence of esophageal dyskinesia, transient relaxation of lower esophageal sphincter tension, and esophageal hiatal herniation, thus leading to an increased incidence of GERD. Obesity also leads to increased intra-abdominal pressure, which increases the likelihood of stomach contents passing through the lower esophageal sphincter [24]. Centripetal obesity also impairs the structure and function of the esophageal epithelial barrier [25]. It has been shown that the accumulation of lipids and their byproducts inside and causes protein breakdown to worsen, dysfunction in the mitochondria to be induced, and a variety of cytokines that trigger inflammation to be produced more often, which can exacerbate muscle atrophy [26, 27]. Sarcopenia is made worse by obesity, which also enhances the chance of mortality, reduces physical functioning, and promotes the infiltration of fat into muscles. [13]. But there is a study finding that people with sarcopenia had a relatively low BMI compared to patients without sarcopenia [28].

5.3. *Diabetes mellitus type 2, T2DM*

The results of two Mendelian randomization (MR) analyses [23, 29] have both suggested that genetic susceptibility to T2DM increases the chance of developing GERD. Mechanisms involved in both are likely to be altered glucose and insulin metabolism in patients with T2DM, which leads to increased vascular endothelial damage and chronic inflammation [30]. Additionally, sarcopenia was more common in T2DM patients than in participants without diabetes by 18% [3]. Patients with T2DM have dramatically lower muscle power and resistance, and poor glycemic control may further lead to reduced muscle mass [31]. In addition, lack of exercise in patients with T2DM leads to muscle atrophy and mitochondrial dysfunction, due to the inhibition of the nuclear receptor's gamma coactivator-1 alpha peroxisome proliferator-activated receptor [32].

5.4. *Smoke*

A cohort Study Shows Smoking Independently Associated with GERD Symptoms [22]. According to a meta-analysis conducted by Larsson and colleagues [33], there is a correlation between smoking habit and a higher chance of getting reflux disease of the stomach. The outcomes of two other MR analyses [23, 34] similarly come to the conclusion that genetic predisposition to start smoking is linked to a higher chance of developing GERD. Because tobacco prolongs gastroesophageal acid clearance and decreases lower esophageal sphincter pressure [35]. MR analysis by Park et al [36] showed that there was a strong correlation found between lower appendicular lean mass index and increased genetic vulnerability to smoking and slower walking speed in the sarcopenia phenotype and was not significant for the grip strength phenotype. Smoking leads to skeletal muscular dysfunction by reducing the amount of oxygen that reaches the mitochondria, where long-term illnesses can cause sarcopenia and muscle atrophy [36]. Simultaneously exposure to cigarette smoke extracts leads to a rise in muscle growth inhibitors and a decline in the rate of myosin synthesis [37].

5.5. *Psychological factor*

According to a meta-analysis conducted by Zamani and colleagues [38] indicated that genetic susceptibility to anxiety and depression was related to a higher chance of developing GERD. The outcomes of MR analysis by Ruan et al [39] indicated that genetic predisposition to depression was related to a higher prevalence of GERD. Because psychological factors like sensitivity to esophageal stimulation alter esophageal dynamics and lower esophageal sphincter pressure, people who suffer from combined psychological disorders often experience pain from low-intensity esophageal stimulation [40]. Additionally, the use of medications like tricyclic antidepressants can cause a decrease in esophageal

sphincter lower pressure [40], which in turn can increase the incidence of reflux episodes [41]. According to both meta-analyses [42, 43], depression was independently correlated with sarcopenia and was more common in sarcopenia patients than in the overall population. Sarcopenia can result from depression, which can also cause a decrease in regular social interactions. People with sarcopenia may also become disabled or become less active due to a loss of muscular mass and strength [43].

6. Conclusion

In conclusion, although GERD and sarcopenia are two completely different diseases, existing research suggests that there is a link between the two in terms of epidemiology and pathophysiology, especially in terms of inflammation, obesity, and psychiatric factors. The treatment of sarcopenia has a protective effect on GERD, and there are also Common potential treatment modalities such as lifestyle improvements like weight loss, smoking cessation, etc. Consequently, clinicians need to pay enough attention to this. It is hoped that the combination of exercise and taking vitamin D as needed with conventional treatment will enhance the patient's prognosis.

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Omics techniques reveal the pathogenesis of microbial communities in asthma

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Abstract. The presence of airway hyperresponsiveness in asthma provides the basis for constriction in bronchus response to a variety of stimuli, leading to limited airflow, dyspnea, wheezing, and chest tightness, threatening patients' lives. Statistics have shown that asthma is more prevalent in the adolescents and children than in the adults, suggesting that early ecological dysregulation of the gut and airway microbiome contributes to the occurrence of asthma, especially in the preschool years' children. With the development of high-throughput sequence technologies such as genomics, it has become possible to study the association of pathophysiological processes of allergic reactions and ecological dysregulation using the multi-omics techniques. In this review, the author first briefly introduces the classification of asthma, summarizes the pathogenesis of microorganisms in asthma, providing a new direction and ideas for the research of the pathogenesis, diagnosis, and treatment of asthma.

Keywords: Asthma, Omics, Microbiome, High throughput sequencing

1. Introduction

As a relatively common bronchial disease, hyperresponsiveness in airway is an important clinical feature of asthma, serving as the foundation for bronchoconstriction in response to a range of stimuli, which can result in a number of life-threatening symptoms include dyspnea, wheezing, chest tightness, and airflow restriction [1]. Statistics have shown that asthma is more prevalent in the adolescent and child population than in the adult. And it is well documented that early ecological dysregulation of the gut or airway microbiome predisposes children to follow the trajectory of asthma [2]. This is because viral and bacterial infestation can contribute to wheezing, especially in the preschool years, and infestation of the airways by *rhinoviruses* (RVs), *respiratory syncytial viruses*, and *parainfluenza viruses* is a common contributing factor in the development of wheezing and acute asthma, and the overabundance of certain bacteria has been shown to be associated with episodes of wheezing, such as *Haemophilus influenzae*. The primary cause of early airway structural changes, which may ultimately result in the early onset and exacerbation of airway remodeling, is genetic defects or variants that sensitize airway epithelial cells to environmental stimuli. Recurrent viral infections can also induce and prolong airway hyperresponsiveness.

The early sign of asthma often manifests as wheezing. While common respiratory infections are known to stimulate type 2 inflammation, allergic sensitization usually precedes wheezing triggered by infections. Interestingly, most children who suffer from wheezing due to viral infections in their early

childhood and preschool years do not progress to develop chronic asthma later in life, suggesting that further exploration is needed regarding the association and mechanisms between infections and asthma development. The high-throughput genomics technologies has become possible to study the biochemistry and pathophysiology behind allergic reactions, presenting application in clinic through identification of new markers and analysis of enrichment functions [3]. In this paper, the author first briefly introduces the classification of asthma and summarizes the pathogenesis of microorganisms in asthma, focusing on the analysis of new advances in high throughput sequencing technology in the pathogenesis and diagnosis of asthma in recent years, providing a new direction and ideas for the pathogenesis of asthma and diagnosis and treatment of asthma research.

2. Classification and phenotypic characteristics of asthma

The asthma is classified by the clinical features, such as the recurrent episodes of wheezing with shortness of breath, which are associated with exposure to allergens, cold air, exercise, etc., named as typical asthma. In contrast to typical asthma, the main manifestations of atypical asthma are that the coughing and chest tightness often occur at early morning or mid-night, strenuous exercise, or exposure to cold air can be triggered by the symptoms of the aggravation of the asthma. According to the triggered factors, the asthma can be divided into different classification from inducing factors such as allergy, exercise, and drug. Allergic asthma occurs in the allergic population, mainly triggered by allergic substances, who is accompanied by allergic rhinitis and other allergic diseases with symptoms of sneezing, runny nose, cough and chest tightness and other symptoms. Exercise asthma is mainly triggered by exercise and the onset of this type of asthma is usually rapid. Pharmacologic asthma is mainly caused by the intake of aspirin enteric-coated tablets or beta-blockers, which is usually characterized by recurrent episodes of shortness of breath, chest tightness and cough. Occupational asthma is mainly caused by frequent exposure to synthetic fibers or adhesives at work, with its main symptoms of chest tightness and coughing during or after hours of work, in addition to rhinitis or conjunctivitis, which are relieved after leaving the workplace.

3. Omics techniques in asthma

3.1. Omics techniques reveal changes in asthma pathology

MicroRNA belongs to non-coding RNA with 18-22 nucleotides that plays a regulatory role in inflammatory diseases. Anshul Tiwari's group conducted a study sequencing small RNAs from mild-to-moderate asthma children (n=398) using the baseline data from seasonal asthma controls. They assessed the impact of allergen sensitization status determined using baseline skin prick tests. Initially, they utilized DESeq2 to identify seasonal differences in miRNA expression, followed by regression analysis to investigate associations between allergens sensitization and differentially expressed miRNAs (DE miRs), eventually revealed 52 miRNAs out of 81 DE miRNAs. Subsequently, they delved into the association between these miRNAs and seasonal allergen sensitization using skin prick tests, finding two miRNAs showed significant associations with allergic symptoms among identifying 26 miRNAs. Interestingly, let-7d-3p exhibited a potentially protective effect against asthma, while miR-328-3p was detrimental to asthma of mulberry trees. Furthermore, the authors conducted an enrichment analysis of target genes regulated by DE miRNAs, revealing significant enrichment in pathways such as Rap1, Ras, and MAPK signaling pathways according to DAVID analysis [4]. This suggests that miRNAs may be involved in asthma by influencing the above signaling pathways.

Regarding the relationship between type 2 biomarkers and the development of asthma, one research group performed a high-throughput transcriptome analysis using 738 whole blood samples from patients suffering from severe asthma with high/low T2 biomarkers. A large amount of RNA-seq data was obtained from 301 participants' blood samples, and unsupervised cluster analysis was first performed to identify 2 clusters. Because patients with high T2 biomarkers had low blood eosinophil counts, were more symptomatic, and were more likely to receive oral corticosteroid (OCS) therapy.

Therefore, the authors identified 2960 and 4162 DEGs using the principle of OCS stratification in differential gene expression analysis. 2960 genes were adjusted by subtracting the OCS signature genes, and 627 genes remained differential involving the biosynthesis of polyidene-bisphosphate oligosaccharides and assembly of the RNA polymerase I complex. However, no stable DEGs were associated with patients with low T2 biomarkers after a trial in clinic, whereas many DEGs were associated with elevated T2 biomarkers, and 15 DEGs among them increased independent of patient symptoms [5].

In addition, high-throughput related sequencing analysis contribute to identify and classify asthma, such as the method of Tape-strips, which is a minimally invasive method of skin sampling that can be used to evaluate a wide range of skin disorders used for asthma detection. Del Duca E et al. collected three groups samples of moderately allergic asthmatic (MAA) children, severe allergic asthmatic (SAA) children and healthy group controls (HC). Subsequently, 1113 and 2117 DEGs were identified in MAA and SAA patients, separately. To distinguish asthma patients from HC using transcriptomic biomarkers, these DEGs were associated with asthma-related symptoms, such as exacerbation rate, lung function of FEV1. Significant downregulation of epidermal transcriptome biomarkers were identification including cell adhesion, terminal differentiation, and lipid biosynthesis/metabolism. The Th1/IFN- γ pathway were enrichment through gene set variant analysis. The genes of G protein-coupled receptors, TGF- β /ErbB signaling-associated proteins up-regulated in both MAA and SAA patients. Skin transcriptome biomarkers were associated with annualized exacerbation rates and lung function parameters, such as TSSC4-FAM212B discriminating asthma from HC with 100% accuracy [6].

3.2. *The microorganisms contribute to the pathogenesis of asthma*

Microbial homeostasis plays a key role in asthma development although the effects of indoor metabolites on health have not been understand clearly. Yu Sun's group collected classroom dust from 24 schools in three locations of distant areas, and performed culture-independent high-throughput microbiome and non-targeted metabolomic/chemical analyses through surveying of a 1290 asthma students. Results revealed significant differences in the prevalence of wheezing between schools at each study center, which could be explained by individual characteristics and air pollutants. Large-scale microbial variability was observed among the three centers with different potentially protective organisms through identification 2633 metabolites and chemicals. Many metabolites were enriched in the low-surge colonies, such as their derivatives. Neural network analyses showed that indole derivatives coexisted with potentially protective microbial taxa, such as *Actinobacillus sphaericus*, *Fischerella sphaericus*, and *Tropezia*, suggesting that these microorganisms may influence health status through the release of indole metabolites. A number of synthetic chemicals are enriched in high wheezing schools, suggesting that they have an aggravating effect on asthma [7].

Microorganisms have an important impact on asthma development, but the impact of fungi on asthma is still poorly understood. Samuel J. Cochran and his team set out to investigate the correlation between fungal diversity and the prevalence of asthma within neighborhoods. Their objectives were to examine household fungal diversity and identify particular fungal species, contributing valuable insights into the environmental factors influencing respiratory health in urban settings. First, author recruited 7-8 year old (n = 347) children living in neighborhoods with high (11-18%) and low (3-9%) asthma prevalence. Subsequently, the authors sequenced fungal communities in floor dust, found that the building type and the presence of bedroom carpet influenced the prevalence and diversity of neighborhood asthma. Additionally, a subgroup of 140 asthmatic children underwent follow-up until 10-11 years of age to assess asthma persistence. Among the 7-8-year-old asthmatic children, it was observed that *Shannon's* fungal diversity showed a negative correlation with frequent symptoms of asthma at that age and asthma persistence into later years. Despite analyzing individual fungal species, no significant associations with asthma outcomes were noted after adjusting for false discovery rates [8]. This suggests that fungal-induced asthma is not dominated by a particular group of fungi, but rather a combination of various fungal interactions with the environment and the organism.

In addition, the Leonardos Mageiros research group summarizes the results of recent studies on the relationship between viruses and asthma, as described above, viruses are strongly associated with the asthma progression, but the impact of virus-host interactions on asthma is still unclear, so the objective of this paper is to investigate the possibility that allergic diseases may be influenced by interaction between virus and host. However, little studies investigating the virome suggests that future studies will require prospective replicated sampling designs to reveal the impact of the virome on asthma development and its interactions with the microbiome and immunity [9]. The application of omics techniques in asthma are listed in Table 1.

Table 1. The omics data in asthma.

Omics data in asthma	Examples
Prediction	The fungal diversity is negatively correlated with the prevalence of asthma [9].
Diagnosis	The TSSC4-FAM212B was able to discriminate asthma from HC [6].
Pathogenesis	Many DEGs were associated with elevated T2 biomarkers [5].
Treatment	The miR-let-7d-3p is a candidate in therapeutic asthma, whereas miR-328-3p exacerbates in allergic asthma to mulberry trees [4].

4. Summary and prospect

High-throughput sequencing and its related analytical techniques contribute to identify new genes responsible for asthma pathogenesis and reveal the mechanisms of microbial roles in asthma development and disease progression. For example, researchers have found that miR-328-3p are significantly associated with symptoms of seasonal asthma. In addition, transcriptome analysis by collecting microorganisms in large samples from multi-regions and multi-centers can provide new insights into the prediction, prevention and precise control of asthma. Moreover, in recent years, high-throughput sequencing has further explained the effect of fungal diversity on asthma occurrence, suggesting that the diversity of fungal species can reduce the incidence of asthma.

With the development of omics technologies, it is possible in the future to find more precise ways to reveal the pathological changes in asthma, and to show the influence and mechanisms of microorganisms in asthma by continuously optimizing clustering and sequencing techniques. In the urban environment, the incidence of asthma is higher in the locations of lower fungal diversity. Future needs to utilize the methods and approaches of prospective studies to reveal the roles and mechanisms of viral groups in causing allergic diseases.

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Research on the impact of congenital joint activity and flexibility on the development of dancers

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Abstract. The importance of range of motion and flexibility in dance training is self-evident. The article aims to solve the typical training problem of dancers due to the growth difference between joints and muscles under various conditions. Common hip injuries in dance training include iliopsoas muscle strain, synovitis, etc., which may be caused by sudden twisting or overextension of the hip joint. To improve flexibility, auxiliary training such as Pilates has become the first choice for dancers, by strengthening the toughness and stretching of fascia and muscles, reducing the risk of hip friction and injury. In auxiliary training, the training of antagonist muscles is crucial, by transposing a set of antagonist muscles to exert force, effectively improving leg flexibility while maintaining joint stability. In addition, the activation of the physical function of the trainees before the class is also key, and the warm-up activities are used to increase the level of bursts and reduce the risk of muscle strain. Taking into account the range of motion, flexibility training, and physical function activation, we can provide dancers with a more scientific and effective dance training program to ensure their physical health and performance quality.

Keywords: Congenital joint activity, flexibility, injury, dance.

1. Introduction

In today's era, with the comprehensive growth of dance culture, the increasing attention it receives highlights its significance as an irreplaceable art form. As a carrier of art expression, the dancer will express and transmit emotions with the body. So, the dancer will pass a long period of self-tempering to achieve the level and quality of the art profession. Dancers were asked to enter professional colleges and institutions to start polishing themselves because of such a professional nature. Starting from various conditions, coaches will conduct comprehensive training and development of them. In the art of dance, it values the physical conditions of the students, including height ratio, muscle strength, and physical flexibility. After the investigation and research of Beijing Dance Academy, the author found that most of the requirements are congenital. Although it does not mean that it cannot be achieved by acquired training, it is very difficult. Among these items, the most important thing is the flexibility of the dancer. This is because in the art of dance, whether it is a classical ballet in the West or the ethnic dance produced under the support of Eastern aesthetics, it is very important for the actor's body in the performance. The dance system takes geometric graphics as the aesthetic principle, and through expansion and suppression of itself, thereby achieves a composition that conforms to aesthetics [1]. With the expansion of the body's

range, the emotions passed to the audience will effectively improve. The flexibility enables the dancers to enlarge their vision from the perspective of the audience.

In the context of the continuous development of the dance art system, in addition to paying attention to the final artistic output of dancers, people pay more attention to the training methods with dancers. People hope to ensure the scientific trainee in this unique profession that needs to be expressed in the body. Through theoretical understanding and explanation of dance performance, skills, and physical exercise methods. As a result, the subject of dance science is formed to help students and coaches improve the quality of dance performance correctly and scientifically. Each individual's physical adaptation of dance art is different.

There exists a question on how flexibility can be scientifically assessed to determine its impact on the quality of dancers. Congenital joint relaxation, which seems to be an advantage in this profession, really makes dancers gain more than disadvantages in subsequent training.

This article aims to discuss the advantages and disadvantages of the future development of congenital joint activity and flexibility. In the previous article, it was mentioned that professional dance colleges will use several standards to measure whether the trainees are suitable for learning dance. The age of the students is generally between nine and ten years old. The inevitable injury in the training of strength is also a problem that the parents and coaches of the trainees pay attention to.

This study will take the analysis of the possibility of the hip joint as an example to conduct an in-depth analysis of subsequent changes caused by the congenital differences in joint activity and flexibility. The article uses the basic measurement of range of motion (ROM) to find the relationship between joint activity and flexibility [2]. The author hopes to evaluate the dancers through dance science and human science. The conclusions may be suitable for judging whether the trainees are suitable for professional dance learning from a new perspective, thereby reducing the possibility of injury. Under the requirement to meet the stretching needs of different types of dances, it will not cause damage under excessive relaxation of body ligaments and joints.

2. Congenital Joint Activity and Flexibility

In the entrance examination and review of dancers in China, admissions teachers need to conduct a comprehensive inspection of them, basic proportions, flexible inspections, and coordinated tests through multiple rounds of examinations. Young students are in a period of development and often cannot accurately judge whether they can fully meet the follow-up standards of dance training and aesthetic requirements. In the current training system, no regular physical indicators are measured in their physical growth after the students enter the school. The various indicators will be unpredictable in subsequent training, especially the different uncertainty of the hip joints, causing the dance posture, the amplitude of the bouncing, and the opening and closing of the limbs. Therefore, it is necessary to combine dance training in human science and sports science to provide accurate data for the improvement of students in the later period.

2.1. Definition and measurement of congenital joint activity

In dance training, students are often required to reach and adduct their joints, which requires coaches and students to be very clear about each joint activity. It is generally observed that joint activity has clinical importance when dealing with muscle-skeletal conditions [3]. The degree of joint activity is divided into passive activity and active activity. Due to the large joint activity, some students find it relatively easy to complete specific actions, and vice versa.

Dance is based on human movement, and the purpose of joint activity measurement is to clarify the most basic assessment items implemented by human movement, which will effectively improve the predictive predictability of dancers to develop physical training and other training. The joint activity of dancers is often greater than ordinary people, so it is necessary to measure and evaluate some trainees with limited joints. Human joint movements are limited by motion planes (sagittal planes, coronal plane, transverse planes) and movement axes (sagittal axes, coronal axes, transverse axes, vertical axes), and have high standards in measurement accuracy [2].

2.2. Definition and measurement of flexibility

Dancers' flexibility is the main factor that supports their actions. Flexibility is closely related to age, body shape, muscle quality, etc., and the individual differences of flexibility are much greater than the activity of the joints, and they are specific [4]. Flexibility refers to the ability of muscles and ligaments to be able to extend, stretch, and curve freely when they receive external force. Students will bring the action of actively stretching the leg muscles and ligament to complete the high stretching action. The conventional measurement method will no longer be suitable for dancers' flexibility measurements. Except for conventional body flexion, standing or sitting partial extension, the Motion Analysis System, Electromyography (EMG), etc., should be added. To measure dynamic flexibility for dancers, and provide detailed data and analysis.

2.3. The relationship between congenital joint activity and flexibility

The joint active activity mentioned earlier occupies the vast majority of dance movements, and it is a joint movement generated by the muscles of each student's individual. In active activity, muscles control joint movement through contraction or abduction, which is associated with flexibility.

Congenital joint activity is closely related to flexibility, and most of them are positive. Good congenital joint activity usually corresponds to the better flexible level, because the joints have a large range of exercise at birth or during growth. When dancers are limited in joint activity (but still greater than ordinary people), the flexible training effect will be reduced, and muscle and ligaments cannot be fully stretched within the range of joint activity. A compensation movement will occur in the unable-reached action, which may lead to a bad movement mode. Such students can improve this problem as much as possible through other auxiliary training, which will be mentioned later. Even if their congenital joint activity is limited, they can enhance the stretching ability of muscles and ligaments through flexibility-assisted training, thereby improving the flexibility level.

3. Research and Analysis of Dancers' Hip Injury

3.1. Hip joint anatomy and motion characteristics

The hip joint is one of the largest joints of the human body, and it is also the most commonly used joint of dancers. It connects the hip and femoral bone, which plays the role of supporting the body up and down. Hip joints distribute many muscles, including iliopsoas, gluteus maximus, quadratus lumborum, quadriceps, etc. They participate in the exercise of dancers to varying degrees. Whether it is a student or an experienced dancer, they should give priority to grasping the problem of exertion of the muscles around the hip joint during training, and this will largely help them complete the connection between the leg movements and the upper body.

Students should first understand the maximum passive activity of their hip joints, and they can measure their exterior angle values in a supine posture. The reference range of the normal hip outreach activity is 0° to 45° , and the upper part of the femoral neck is supported at the top of the acetabular fossa at the extreme posture. At this time, you should pay attention to the pelvic tilt, that is, the compensation movement [2]. For dancers, such a small range of activity is not enough, so the professional characteristics of dance make students need to master different combination methods. For example, the dancers need to control the stretching of the legs more than 90° . This requires not only active outreach, but to combining multiple ways of force, which is indispensable. When the passive activity of the outreach reaches the limit, the front end of the femoral neck is positive facing the acetabular fossa through the hip joint. After avoiding the hip-opening fossa, the exterior range can be significantly increased. In cooperation with the dancer's trained iliopsoas and gluteus maximus, it will be able to reach the visual-super stretch of the dance aesthetics.

3.2. The effect of congenital joint activity and flexibility on hip joint injury

Injury is the most common and inevitable in the dance profession. Pulled muscle caused by the problem of exertion, or bone damage caused by lack of ability when completing difficult techniques, etc., often

plagued students and coaches. Among them, the most common injury occurs in the hip joint. In the previous article, the hip joint as the most widely used part of the dancer has assumed a stable and load role. Congenital joint activity and flexibility have great guidance for injury to this part. When the hip activity is limited, high-intensity practice, external rotation, and flexibility training, most of them will be counterproductive. The cycle of biomechanics of functional disorder expounds on the problems faced by the dancer during the injury stage. Due to the continuous stimulation of the hip joints during the training, it has caused changes in biomechanics and painful or structural injuries [5]. After that, the dancers instinctively avoid pain points, which reduces muscle function. The hip joint will further lack muscle protection, resulting in the reactive tension of the dancer, the muscle will be continuously tense, and then the same part of the injury will occur again [5].

As mentioned earlier, limited joint activity is often accompanied by poor flexibility, and flexibility is critical to preventing hip injury. Having muscle stretching ability and elasticity of soft tissue around the hip joint will help reduce the pressure and tension, improve the stability of the hip joint, and at the same time avoid compensation movement to the greatest extent. Dancers' specific flexibility training methods will be explained in the final chapter.

In dance training, there is another type of student-injured hip joint. Their congenital joint activity is very relaxed, providing great convenience and shortcuts for professional training and flexible training. This type of student is the opposite of the former. With the support of a larger joint activity, it can be easier to complete the large dance action. Such students are considered to be in line with learning dance when recruiting dance colleges, and they have reached the standard of dance in both hip outreach, external rotation, or internal collection. Often, those students have suffered a lot of disadvantages in subsequent training. The current teaching system overlooks the follow-up results caused by the large joint activity. This symptom is also known as congenital joint relaxation, which is a congenital disease, often accompanied by congenital ligament relaxation.

The joint ligaments of such students generally lack elasticity, and they usually increase the possibility of joint injury or even dislocation. According to statistics, among young dancers, people with congenital joints relaxation are quite wide [3]. Dance students injured with congenital joints relaxation even more than those of the limited joint activity which mentioned earlier. In dance performances, muscles and bones play a complementary role. After the joint passive activity has a normal range, it will drive the force through the muscles to reduce the friction of pure joint and bones. Students with excessive joints activity usually do not actively or use surrounding muscle tissues in a small amount during training, and their visual extension is still even greater than other students, which is relatively convenient and easy for them. Under the accumulation of training for years, the muscle quality of these students has not received the required growth, especially reflected in the hip joints with high requirements in dance movements. When they are used to driving the stretching movements of the legs through pure bone and joint motion, whether their inner or outer muscle group will not be able to protect the stability and buffer of the hip joint, leading to hip joint wear or dislocation.

3.3. Types and incidences of dancers' hip injury

Hip joint injury is divided into multiple types, and the incidence of dancers is significantly greater than ordinary people. In dance training, the incidence may be affected by multiple factors. It may be caused by the joint movement and flexibility mentioned earlier. If high-intensity training lacks buffer may cause fatigue injury. The author conducts questionnaire surveys of various subjects in the Beijing Dance Academy in China, including ballet, Chinese dance, international standard dance, etc. The age range of the survey is 9 to 18 years old. After the survey and analysis, it was found that under the combination of all dances, the highest incidence of injury site was the hip joint, followed by ankle joints. Therefore, this chapter mainly analyzes the type of hip joint injury.

The most common injury among students is the strain or sprains of the iliopsoas fascia, which accounts for 63 % of the number of hip injuries because it is suffering from greater pressure in training. When students train in dance repertoires, often due to various uncertain factors required, causes sudden bending of hip joints, and excessive stretching of hip joints may also cause strain of iliopsoas fascia. At

the same time, the improper movement made by the students when they did not master the technical requirement, increased the risk of injury to the iliopsoas fascia. The occurrence rate of synovitis often occurs in the early stages of osteoarthritis [6]. Due to frequent daily dance activities and repetitive training, the hip joint will be affected by inflammation. Studies have shown that synovitis is related to poor hip structural function, that is, dancers with limited joint activity may be more likely to have synovitis [7]. Dance movements can increase the pressure inside the joints, and at the same time, the friction of the joint surface may increase, thereby increasing the risk of dancers forming synovitis. The accompanying pain will lead to further limited joint activity. When dealing with dance training, a small number of students will involve high-intensity hip joint movements and heavy pressure. In the case of severe wear, the cartilage on the acetabular fossa and neck of the femoral is harmed. Those students cannot practice jumping and hip joint rotation action with the same intensity after injury.

Hip dislocation and femoral head necrosis are caused by long-term training, there are almost no trainees between the ages of 9 to 18, only individual cases, such as congenital hip dislocation. Congenital hip dislocation is widely increasing among women. Due to the dysplasia of the hip joint during the fetus, the femoral head is not fully embedded in the acetabular fossa. This condition will bring disadvantages to the dancer's career. The congenital hip dislocation has gradually received the attention of the dance profession in recent years. The characteristics of this disease are reflected in the limitation of the patient's hip activity, pain in the outreach and internal collection direction, and has a great impact on the training of dancers. With the growth of the student's body, people with this condition can only reform the hip joint structure through surgery. There is a long-term recovery after surgery. For professional dance students, it is very unfavorable. Femoral head necrosis is more common among adult dancers. In their professional training for more than ten years, oppression and injury caused by hip joints have accumulated to a certain extent. The long-term insufficient blood supply has led to the gradual death of the tissue around the femoral head, forming a serious hip joint problem.

4. The Training and Prevention Strategy of Congenital Joint Activity and Flexibility

For dancers, the fascia and muscles of hip joints and pelvis need to be strong. In the dance, single-legged standing, supporting power leg movement and stretching, or single-legged support as the axis rotation were usually used. The pelvis needs to support quite a large weight in training. In these actions, the weight of the hip joint will be twice of dancer's weight, and the techniques such as jumping need to bear three to four times. Strengthening flexibility can effectively reinforce the toughness and extension of fascia and muscles, provide dancers with dynamic and static stability, and minimize friction and injury at the hip joint as much as possible [8]. Although the joint activity caused by natural bone development cannot be changed, good flexibility can visually improve the dancer's movement range, and use muscles to drive joints to play the highest joint activity range that can be used. In recent years, more and more dance auxiliary training has joined the daily life of the students. Coaches have also tried to enhance the flexibility of the students in the most scientific methods, instead of relying on the most primitive and most injured methods, which is leg pressing. In the process of contacting dance science and human anatomy, students gradually understand the body structure of a dancer, which is suitable for dancers' force.

4.1. Training method and skills

The dancers used the most widely-known auxiliary training, known as Pilates, and they penetrated the course from entry to senior dancers. Its founder was Joseph Hubertus Pilates from Germany, which developed Pilates training in the early 20th century. It is used as rehabilitation training with sports athletes and pregnant women in the early stage. Nowadays, after the continuous attempts and use of the coaches and the trainees of Pilate, it has successfully integrated into the field of dance and has become the best choice for dancers. Pilates is concluded in clinical trials to strengthen the effectiveness of improving flexibility and pelvic stability, and also enhance muscle activity [9]. The coordination of breathing is important for Pilates training. It mainly uses controlling movements and mobilizes multiple

outer and inner muscles together. For dancers, Pilates needs to emphasize the core muscle group, which is the best way to assist dancers in training flexibility.

The muscles are divided into two types in Pilates, which are respiratory muscles and action muscles, and the distribution of them is sometimes overlapped. Breathing is divided into chest breathing, abdominal breathing, and intercostal breathing. It is not right or wrong with each other, suitable for different training movements. Pilates emphasizes the training of core muscle groups, such as rectus abdominis, transversus abdominis, obliquus externus abdominis, obliquus internus abdominis, iliopsoas, and pelvic floor muscles. These inner muscles are responsible for balance and stability when used as respiratory muscles. Provide a larger scope of activity for motion muscle. When the core muscle group stabilizes, it effectively helps the pelvis to reduce weight and pressure, so that the friction of the acetabular fossa and femoral head are reduced. With muscle mobilization, the dancer reduces the heaviest-weight pelvis in the body, making hip joint minimize limitations to activity. It will enhance the possibility of increasing flexibility.

In the auxiliary training for flexibility, antagonistic muscle is the key. In simple terms, antagonistic muscles give active muscles in the motion of resistance. Each group of active and passive muscles in the body is called a set of antagonistic muscles. When active muscles contract, the other passive muscles will relax to ensure exercise. Taking the dancers to control their leg forward stretch of 90° as an example, at this time, the hamstrings and quadriceps are a set of antagonistic muscles. Quadriceps actively lift the legs. As the antagonistic muscle at this time, the hamstrings will be relatively relaxed to maintain the balance of force. However, in auxiliary training, coaches will ask students to train this group of muscles oppositely. The trainees lay their legs in the hands of the coaches, with an angle of 45° to the ground. The student's legs pressed the hand downward, and the coach cooperated with the student's upward resistance for half a to one minute. At this time, the quadriceps became an antagonistic muscle, in a relatively relaxed state, and the hamstrings became active muscles. This kind of training makes a set of antagonistic muscles have a strong contraction and relaxation stage and has obvious activation of muscle and ligaments [10]. The opposing force helps to balance the strength of the legs of the dancer, effectively enhancing the flexibility of the legs without harming the passive activity of the hip joint and maintaining joint stability. In Pilates, students often use special equipment to help them completely different types of training, and the equipment has greatly improved the flexibility of training. The antagonist muscle training mentioned earlier, with the help of the equipment, will not be limited to lying on the flat movement. Using the Pilates bed and the tension belt in the standing position, compared with the former, is more in line with the characteristics of dancing physical movement.

4.2. Precaution and suggestions

Due to the high rate of injury in dancers' training, dance therapy was set up to help the dancers recover quickly after being injured and return to the training class. In addition, letting dancers scientifically master precautions can greatly reduce the incidence of injury and prevent problems before they occur. However, pre-injury prevention has not been paid enough attention, and the corresponding medical assistance investment is limited. If the medical field can be linked to the dance profession, it will prevent potential danger and injury to dancers' movements and training, and also effectively extend a dancer's career, which is very efficient [11]. When integrating the dance profession in the medical field, the most important thing for dancers and coaches is to turn theory into practical training, and successfully apply it to their own body. Sports mechanics and physiology should gradually join, which can be divided into two types: academic lectures and practical courses. In academic lectures, mechanical forces allow students to understand the rationality of movements, and they can be refined into joint kinematics and muscle mechanics. Let the trainees be grasped by analyzing the performance of body mechanics to various parts, the size and direction of the force, and the action point of the body, can reduce the possibility of injury. Although physiology and sports mechanics are solved differently, theory is crossing each other. Let students contact physiology so that they can master how to relieve muscle fatigue and how the respiratory system can adapt to high-intensity training with dancers. Each individual has different optimization methods, which are determined by congenital bone development and muscle

quality. After fully grasping theoretical knowledge, trainees should discuss with teachers and enter the course for practical exercises to truly solve the problems of dance performance and movements. For students, there is a clear and controllable mind for their physical activities during training, which will be the best injury precaution.

The physical function activation of students before class is important, also known as warm-up activities. There are many highly explosive muscle forces in dance training. If students use relatively complete pre-class activation and warm-up, it will effectively improve the outbreak level in the follow-up course and reduce muscle strain [12]. At present, most students are consciously stretching flexibly before class, and some joint outreach and extension can be performed in advance to increase the range of joint activities in the follow-up course. Students are generally lacking in muscle activation. The author takes the hip joint as an example to achieve muscle stability and activation. For the stability of the pelvis, there are two groups of muscles to help balance. Core muscle groups, hamstrings, pelvic floor muscles, gluteus maximus, etc. are responsible for posterior pelvic tilts. Students can activate this part of the muscle group through training such as abdominal and back muscles, squats, and Pilates training. On the contrary, the latissimus dorsi, multifidus muscle, rectus femoris, sartorius, etc. are responsible for anterior pelvic tilts, which can be activated by side-raising legs and waist stretching. After the students complete the muscle activation through this structure, they can consciously complete the neutral position and stability of the pelvis in subsequent training, giving the hip activity and lower limb flexibility in the best motion state.

5. Conclusion

To sum up, the importance of innate range of motion and flexibility in dance training is self-evident. Studies have shown that limited range of motion is often accompanied by poor flexibility, which plays a key role in preventing hip injuries. However, excessive range of motion may also lead to drawbacks in subsequent training, especially in trainees with congenital laxity of range of motion, and may increase the risk of joint dislocation or injury. Therefore, in dance training, it is necessary to comprehensively consider the innate range of motion of the trainees to formulate a personalized training plan, which can not only improve the range of motion but also avoid joint damage. In addition, the hip joint is subjected to greater force and stress in the dancer's body, so special attention needs to be paid to the health of the hip joint during training. Common hip injuries include strains or sprains of the iliopsoas fascia, synovitis, etc., which may affect dancers' training and performance. Enhancing flexibility training, it can effectively strengthen the toughness and extension of fascia and muscles, provide dynamic and static stability, and reduce the risk of friction and injury at the hip joint. By comprehensively considering factors such as congenital range of motion, flexibility training, and hip joint health, dance training can be carried out more scientifically and effectively, providing a better guarantee for dancers' physical health and performance quality. In the future of dance training, the process of development and growth of students' bodies, should be combined with the continuous changes of bone and joint muscles, delve into the corresponding scientific measurement, and then adjust the follow-up targeted training. Under the combination of dance art and medical science, dance science can be very reasonable in traditional dance training. Fully grasp the growth or weakening of students' ability at each stage to achieve reasonable scientific training.

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The impact of population aging on China's health care system and countermeasures

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Abstract. Today, the aging population has already become a common problem faced by many countries, and China is not an exception which has entered the aging society. This problem would bring various impacts on China's social development. This review would focus on the impact on health care system. One is the excess demand for the health care services since the demand of elderly is special, which is different from ordinary patients. Second, with the growth of the number of elderly, the supply of pension insurance funds is reduced but the expenditure is increasing. These has brought great pressure on the health care system of China. After analyzed the impact, the author compared the response of other countries faced with similar problems and provided some suggestions to mitigate the effect of aging.

Keywords: aging problem, population aging, China, health care systems

1. Introduction

Commonly, population aging refers to the increasing trend of the proportion of elderly people in the whole population of a country or region. In general, the definition of population aging involves the increase in the share of the population aged 65 years and older in the total population, and the change in the proportion of the population aged 65 years and older to the population aged 15 to 64 years. As it is mentioned by Liu, a country or region entered an aging society when the proportion of people aged 65 and over reaches 10% or more of the total population, meanwhile, in 2020, China had 191 million people over the age of 65 [1]. The relevant data show that the proportion of people aged 65 and above in China is 15.6 percent in 1999, had become the aging society, which problem keeps still developing [1]. From the line graph, Figure 1, the proportion of elderly people increased year by year, and the researchers also showed that people over 80 years old became the fastest growing group of the elderly population [2].

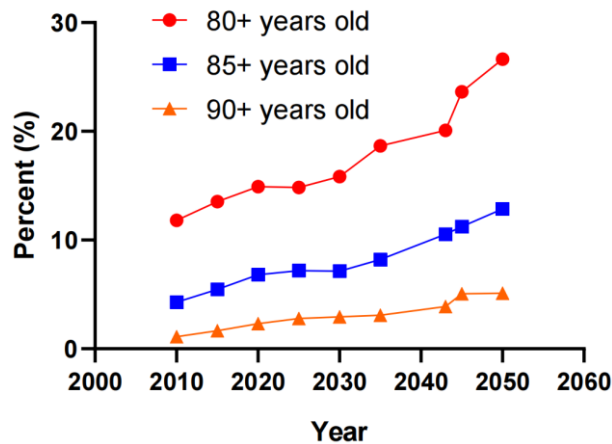


Figure 1. Proportion of the elderly at different age groups in China between 2010-2050 [2].

With the deepening of the aging problem in society, the labor force of China's society will be tightened, and the pension burden will rise rapidly, which will have an impact on China's healthcare service and system. Therefore, it's necessary to raise its attention to this problem and formulate countermeasures in time according to the development trend. This review focused on the impact of the aging population on the medical market in China. The author first claimed on the situation and future development trend of aging in China, then, clarified the impact of population aging in the healthcare system. The third part of the essay introduced the experience of other countries to deal with aging, and the last part was the conclusion and suggestions to control or decrease the impact of aging.

2. The trends of aging population in China

Due to the decreasing birth rate, increasing in life expectancy and the effects of population control policies, the trends of an aging population are becoming more pronounced.

First, the aging problem would show in the growth of the elderly population. As predicted by the researchers, the amount of the total elderly population in China will be about 300 million in 2025, and it will reach more than 400 million and 483 million in 2033 and 2053, separately, and 2/5 of the elderly in Asia and 1/4 of the elderly in the world will come from China [1].

Second, the proportion of the labor force also reflects the aging of China. Since 2012, China's working-age population aged 15 to 59 has increased annually [3]. The rate of reduction is more than 3 million, and the cumulative reduction has been more than 40 million by 2022 [3]. Besides the decline in the total amount, of China's labor population, the proportion of 45-64 years old increased by 7.61% in the nine years from 33.74% in 2011 to 41.35% in 2019 [1]. This demographic shift challenges sustaining economic growth, productivity, and social welfare systems, as there are fewer individuals of working age to support the growing elderly population.

3. The effects of aging population in China

3.1. The influence of population aging on the market of healthcare

The first point that should be illustrated is an apparent impact on the healthcare market. With the growth of the aging problem, the demand for medical services is growing rapidly.

From the perspective of health economics, health could be regarded as a kind of capital of humans. There will be depreciation in the process of capital accumulation, which refers to the loss of assets due to wear and aging and other factors in the use process, thus reducing the actual value of possession, and this problem will also exist in health capital. The growth of age raises the depreciation rate. This could lead to more problems in physical health and increase the demand for health care. The increase in depreciation rate can be reflected in many aspects, for example, the elderly have a higher

depreciation rate of muscle and bone. With the increase of age, the incidence of human muscle and bone-related diseases gradually increases, among which sarcopenia and osteoporosis are more common. This increases the risk of falls and fractures in the elderly [4]. It seriously affects the quality of life of the elderly and increases the demand for medical care [4].

Besides the problem mentioned above, the shortage of health care supply are particularly acute in mountainous areas. The unbalanced development of the economy has led to the fact that the medical security and material foundation in rural areas are not as good as those in cities. Zhu mentioned in the analysis that general township (town) public hospitals and urban street hospitals are mostly “B” type hospitals [5], which admission pressure and complexity of patient health management are much higher than those of urban street hospitals. The demand for medical services is 30,000 to 100,000 people, with fewer geriatric departments and professional nursing staff, and the supply of medical services exceeds the demand, exacerbating this problem because of population aging. Sun’s analyzed and compared the distribution of pension resources in 2010 and 2019, found that the contribution rate of their index of pension resources allocated by population and area in different years was significantly greater between regions than within regions, indicating that the unfair allocation of pension resources mainly happened between regions [6]. Data from the seventh census in 2020, the proportion of permanent rural residents aged 60 and above accounted for 23.81 percent (121 million) of the total rural population, with 5.1% higher in rural areas than in the national average [5]. The degree of aging may be more severe in rural areas than in urban areas. This also exacerbates the shortage of medical resources in these areas.

In addition, population aging is a very special problem, which is different from other common diseases. Aging not only requires medical services but also nursing care. Both the physical functions in terms of hearing, vision, and physical strength and activities such as eating, dressing, toileting, and moving are more difficult for the elderly, tending to rely on others for help and care, thus increasing the overall burden on society to care for them. The old-age dependency ratio, or the number of people aged 65 or older for every 100 people aged 20 to 64, is expected to rise from 13 in 2010 to 45 in 2050 [7]. The acceleration of population aging brings health, social, and economic problems, including reduced health functioning due to disability and chronic diseases, reduced labor supply, and long-term care costs. The long-term care needs of the elderly have become an increasingly important issue. The deepening of aging accelerates the development of a new industry in the medical market, that is, the old-age industry. By the end of 2020, there were about 329,000 old-age care service institutions and infrastructure facilities, with a total of 8.210 million old-age care beds, an increase of 5.9 percent over the previous year [8]. There were 38,000 registered old-age care institutions nationwide, an increase of 11.0% over the previous year, with about 4.882 million beds, and 291,000 community old-age care service institutions and facilities, with 3.328 million beds [8]. It can be seen from the data that China’s old-age service industry is constantly developing and its scale is gradually expanding. However, due to the large population base and the large number of elderly people, the current number is still unable to meet the demand.

3.2. The influence of population aging on the market of insurance

The second part of the effect of the aging problem on the healthcare market is the increasing burden of the medical insurance system. First, the supply of funds for medical insurance decreases. The main way of raising funds for a domestic active system of primary medical treatment insurance is the payment of employers and employees [9]. People who have retired do not need to pay for the premium of the insurance and have the same treatment as those employees. However, the proportion of retired workers increased, only relying on current employees to pay insurance premiums which increased gradually, leading to stress the medical insurance system. Data calculated by China’s Ministry of Human Resources and Social Security and National Healthcare Security Administration, the total expenditure of China’s basic medical insurance fund for employees increased by 3.92 times from 2010 (327.160 billion yuan) in 2010 to 2020 (1283.399 billion yuan) [10]. Technological progress could be one reason because more diseases can be cured by surgery or drug therapy [9], resulting in releasing of

the original compressed medical demand, further increasing the demand for medical insurance which leads to more medical expense.

4. The countermeasures of aging population in other countries

Except for China, many countries are facing the same problem and are experiencing aging populations, such as Japan and Australia. In response to the challenges posed by population aging, Japan and Australia have implemented various policies and strategies to address the multifaceted needs of their aging populations.

4.1. The strategies to population aging in Japan

In Japan, the proportion of people with age over 65 had reached 27.2% of the total amount, then continue to grow to 38.4% by 2065 [11], posing great challenges to the whole society due to the demographic change. The Japanese government has been aware of this problem and has been constantly adjusting relevant medical policies. To solve the impact and problems brought by the change in population structure, studies have shown that in 1961, Japan achieved universal coverage of UHC, and then entered the second phase of expanding social security benefits, for instance, they published a law that supported the elderly people, subsidized them when needed to pay for the health care services. The third step of the solution of Japan is enhancing sustainability. Through the implementation of the long-term care insurance (LTCI), the structure of the social security system for the elderly was completed, including health care insurance for illness, pensions for livelihood support, and LTCI for long-term disability [11]. To guarantee sustainable development, a key aspect is the promotion of healthy aging through preventive health initiatives and health promotion campaigns. These efforts underscore the importance of maintaining the physical and mental health of older adults, helping to improve quality of life and reduce health care costs over the long term. In addition, Japan has invested in long-term care services and infrastructure to support older people with functional limitations or disabilities, including the implementation of community care programs and the expansion of home care services [11].

4.2. The strategies to population aging in Australia

Australia, like Japan, has also implemented a range of policies and initiatives to address the challenges of population aging. Long-term care services and insurance have been set in Australia in the early times. However, with the development of the aging problem and society, the needs of elderly people changed. The Australian government accurately judged and analyzed the changes in insurance policies and long-term care services related to the elderly in line with people's needs, which is also the success of Australia in coping with population aging. By employing comprehensive assessment tools, the specific needs and preferences of elderly people can be ensured, which could increase the efficiency and result of long-term care [12]. Moreover, Australia's aged care system emphasizes consumer choice and empowerment, enabling older adults to make informed decisions about their care and support services [13].

5. The suggestions for aging population in China

As the aging problem continues to deepen, China should timely adjust its medical system according to the development of the problem, draw on foreign experience, formulate and implement relevant policies, alleviate the impact of population aging on the medical system, and support the health of the elderly population.

The first is to increase the supply of medical services, firstly investment in Healthcare Infrastructure, particularly in rural and underserved areas. Meanwhile, the development of AI can also alleviate the pressure on the medical system to a certain extent. For instance, after a large number of medical data, AI will assist doctors to read CT images and early cancer screening. Research has shown that in July 2017, Ali Health and Wanliyun released the medical artificial intelligence system "Doctor You", which can read 9,000 CT images within half an hour, which is thousands of times more efficient

than human beings [13]. With the development of its ability to learn, the number of things that AI could help will greatly increase, which may reduce the burden on doctors and relieve the pressure on the health care system.

Second, the government needs to more accurately judge the needs of the elderly. As mentioned before, the needs of the elderly for medical resources are different from those of ordinary patients, such as increasing the cultivation of nursing talents. China has intensified the training of medical personnel to improve their professional level and service quality. At the same time, it should encourage the introduction and training of more medical professionals to meet the growing medical needs of the elderly.

Third, China should continue to improve its medical insurance system. Literature describes the current situation of regional imbalance in medical insurance in China [9]. Expand coverage and increase reimbursement rates to reduce the burden of medical costs for the elderly. This will help to ensure that the medical needs of the elderly are met, and it is also necessary to improve the accessibility and equity of medical services and ease the pressure on medical expenditures of the elderly in remote areas. Besides the insurance policies, China should also pay more attention on the investment management of pension funds. The public investment channel should be expanded and the government should improve the policy of transferring state-owned capital to enrich pension funds, so that pension insurance funds can effectively maintain and increase their value, and properly deal with the financial pressure of an aging population [1].

6. Conclusions

Population aging is a pressing issue in China, shown by a significant increase in the proportion of elderly individuals aged 65 and above. This demographic shift poses various challenges to the healthcare market, including a growing demand for medical services and a strain on healthcare resources. As the elderly population continues to rise, the burden on the healthcare system increased, exacerbated by factors such as the depreciation of health capital with age, shortages of healthcare supply in rural areas, and the increasing need for long-term care services. Furthermore, the aging population has implications for the healthcare insurance market, leading to a decrease in the supply of funds and an increase in expenditure. The burden on the medical insurance system is further compounded by technological advancements and the rising cost of medical care. In response to these challenges, countries like Japan and Australia have implemented various policies and strategies to address the needs of their aging populations. For instance, similar to Japan, China should also focus on achieving universal health coverage in future, promoting healthy aging, and investing in long-term care services and infrastructure.

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Effects of different types of music on spatial memory in mice

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Abstract. A preliminary investigation was conducted to find out whether different types of music have an effect on spatial memory in adult mice. Thirty mice were divided into six groups, each half male and half female, namely, female pop group, male pop group, female classical group, male classical group, female control group, male control group, and kept in cages and fed freely. There was a reduction in latency time in the musical group compared to the control group. There was a significant difference between the classical group and the control group ($P < 0.05$), while there was no significant difference between the pop group and the control group ($P > 0.05$). There was an enhancement of spatial memory in mice by classical music and no statistically significant enhancement of spatial memory in mice by popular music.

Keywords: Music, spatial memory, Morris water maze.

1. Introduction

The history of music can be traced back to the early days of human civilisation. The music of different periods has different artistic characteristics, but also conveys the emotions and ideas of people in different periods. After discovering that music has an effect on emotions, people often use music for therapeutic purposes. In 1995, scientists Rauscher and Shaw GL found that temporary listening to Mozart's music can improve spatial cognitive ability, and put forward the famous "Mozart effect" [1]; Hu Jingjing and other experimental research found that TchE and MAO in the brains of mice in the Liangzhu music group were significantly reduced compared to the control group, which signified that the mice's brain was not as strong as that of the control group. In 2013, Chan, Alice S. and Helen Tager-Flusberg concluded that music has a positive effect on brain function and can promote children's language ability and cognitive development [3]; the types of music that have been developed so far have been very diverse, so we decided to select two of the most well-known and popular genres for this study. In this study, we decided to choose the two most familiar and popular types of music, pop music and classical music, to investigate the effects of these two types of music on the spatial memory and activities of mice, in order to lay a theoretical foundation for the future guidance of students' education and patients' rehabilitation and other practices.

2. Materials and methods

2.1. Materials

Thirty healthy adult Kunming rats, weighing 35-55 g, half male and half female, were purchased from the Animal Experiment Centre of Qiqihar Medical College.

Morris water maze; Morris Animal Behavioural Experiment Software; sound-proof box; classical music (Piano Sonata No. 14 in ascending c minor, Moonlight op. 27.2); pop music (Parade), and the intensity of the background music was controlled to be 60~70dB.

2.2. Methods

2.2.1. Clusters

The experimental animals were randomly divided by sex into female control group (n=5), male control group (n=5), female classical music group (n=5), male classical music group (n=5), female popular music group (n=5), male popular music group (n=5)

2.2.2. Intervention

The small speakers were placed in the soundproof box, and the sound volume meter measured 60~70 dB in each cage box when the speakers were turned off, and 30 dB outside the cage box, and 60~70 dB in the cage box when the music was played, and the mice in each group did not interfere with each other when they received the music stimulation. Every day from 9:00 am to 11:30 am and from 15:00 pm to 17:30 pm, the speakers in the cages of each music group played the corresponding music, and the blank group placed the same speakers but kept them turned off, and the water maze test was conducted at the end of each 6-day cycle. A total of three tests were performed, during which the mice were fed and watered freely. The musical frequencies of the two pieces of music are shown in Figure 1.

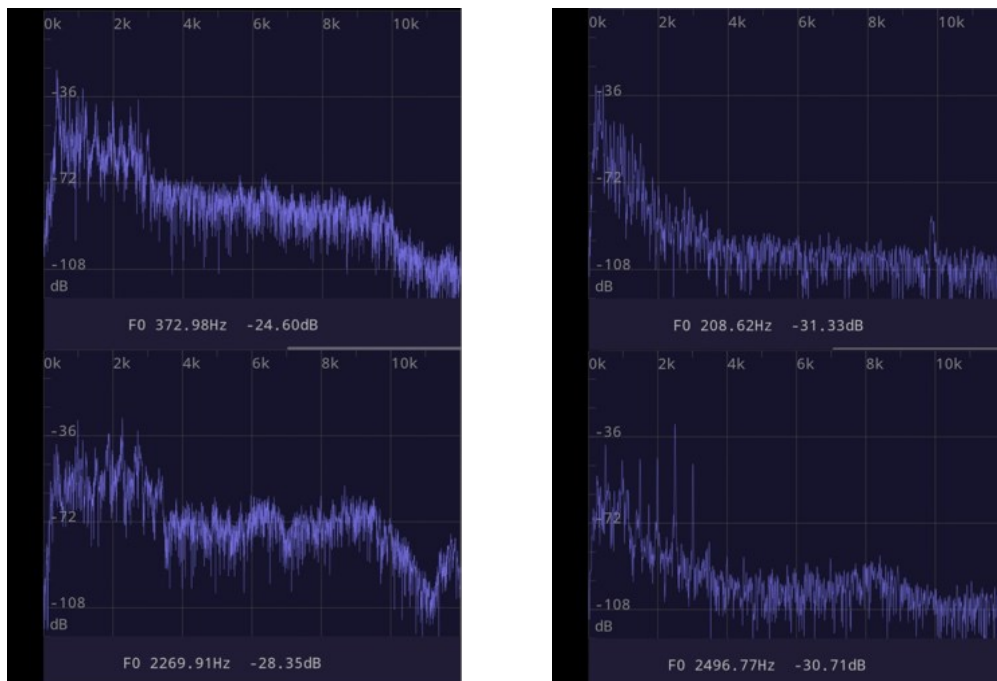


Figure 1. The musical frequencies of the two pieces of music. (Photo credit: Original)

2.2.3. Morris Water Maze Test

The musical stimuli were kept constant during the test. Fresh water was added to the labyrinth, and the water temperature was controlled to be between 18°C and 22°C, with the water surface about 1 cm above

the platform so that the platform could not be seen by the naked eye. The maximum latency of the automatic recorder was set to 90 s. The localisation navigation experiment was conducted at 9:00 am on the 7th day of each cycle after 6 d of intervention. Mice were placed into the pool with their backs facing the platform from four entry points in the east, south, west and north of the pool, and the time taken for the mice to locate and climb up to the platform, i.e. the latency to escape, was observed and recorded. mice that could not locate the platform within 90 s were guided to the platform and remained there for 10 s. The test was conducted four times consecutively.

2.3. Statistical analysis

The experimental data of each group were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$), and the data were analysed using repeated measures analysis of variance (ANOVA), a statistical software, and the difference was considered significant at $P < 0.05$. The results are shown in Table 1

Table 1. Average latency of water maze ($\bar{x} \pm s, n=10, s$)

Group	1st day	2st day	3st day
Control group	51.21 $\pm$ 15.18	50.33 $\pm$ 17.55	51.17 $\pm$ 16.37
Classical group	50.93 $\pm$ 23.35	41.17 $\pm$ 21.74	29.11 $\pm$ 15.42
POP group	44.09 $\pm$ 21.96	46.32 $\pm$ 17.59	44.54 $\pm$ 21.48

3. Result

In the positioning navigation experiment, the latency time was shorter in the music group compared to the control group and became shorter in the classical group as the training time increased. There was a significant difference between the classical group compared to the control group ($p < 0.05$), while there was no significant difference between the popular group compared to the control group ($p > 0.05$).

4. Conclusion

Learning and memory are complex processes, and it has been shown that the establishment of short-term memory is associated with an increase in synapses, with serotonin leading to an increase in presynaptic cAMP, which activates the pka and leads to synaptic enhancement through the release of augmentative transmitters generated by the connectivity mechanism; long-term memory persists through the growth of new synaptic connections, a structural change that parallels the duration of behavioural memory. As memories fade, these connections contract over time [4].

Music, as an integral part of our daily lives and studies, has not only been limited to the role of appreciation and enrichment, but also has therapeutic effects. It has been found that music plays a therapeutic role in psychosomatic and physiological diseases, and has an important role in assisting in the treatment of adverse emotions such as tension anxiety depression and pain [5]. Not only that in the experiment also found that music stimulation can improve the spatial memory ability of rats ,enhance the hippocampal neuron NMDA receptor and encoding NMDA receptor mRNA expression [6].

The results of this experiment showed that the spatial memory ability of mice in the classical group was significantly improved compared with that of the control group, which indicated that classical music promoted the development of related areas in the brain to a certain extent, confirming the enhancement effect of classical music on spatial memory. There was no significant difference between the pop group and the control group, and this result does not confirm that pop music can enhance spatial memory. The music tracks chosen in this study are few, and only a preliminary exploration of different music genres was made, which has certain limitations, and the subsequent experimental study can be conducted by increasing the music tracks of various genres.

Authors contribution

Binying Fu, Jiale Zheng made the same contribution. Binying Fu: Introduction. Jiale Zheng: Conclusion.

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Study on antibody-conjugated drugs and targeted therapy for triple-negative breast cancer

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Abstract. Statistics show that, second only to lung cancer, breast cancer is now the most common type of cancer worldwide. Compared to other forms of breast cancer, triple negative breast cancer (TNBC) has a worse prognosis, extensive metastases, and poor efficacy, making treatment more difficult. antibody-drug conjugates (ADCs), have become a hot topic in targeted tumor therapy research. Gosa tuzumab is the first drug approved for advanced TNBC treatment globally. However, challenges remain in the clinical use of ADCs, such as limited delivery of ADCs to target cells and suboptimal anti-tumor effects. This article analyzes targeted therapy methods and ADCs for TNBC, summarizes current market gosa tuzumab and ongoing clinical trials for ADCs drugs, providing a reference for further study on TNBC treatment with ADCs. Nevertheless, issues like drug resistance, adverse reactions and combination strategies with other chemotherapy inhibitors for TNBC treatment warrant further investigation in future clinical trials.

Keywords: Antibody-conjugated drugs, targeted therapy, triple-negative breast cancer.

1. Introduction

According to statistics from the International Cancer Research, it is projected that in 2020, there will be over 2.3 million new cases of breast cancer worldwide, making it the second most common cancer after lung cancer. Approximately 90% of these cases occur in biological women, posing a serious threat to human health [1]. Breast cancer can be classified into four main types: Luminal A, Luminal B, HER2-positive, and TNBC. TNBC is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2) expression as determined by immunohistochemical examination [2]. TNBC is highly aggressive with a poor prognosis and tends to metastasize distantly [3]. The etiology of TNBC remains unclear; however, factors such as age, endocrine status, and family history are considered risk factors for its development. Due to high tumor mutation rates and challenges in studying disease mechanisms, TNBC is more difficult to treat compared to other subtypes of breast cancer.

There are currently five classes of drugs used for treating TNBC. The first class consists of chemotherapy drugs which serve as the cornerstone treatment for TNBC; however they often come with significant side effects due to their cell cycle-specific anti-tumor properties. The second class includes immunotherapy drugs like cyclophosphamide injection which work by enhancing the body's immune

function to eliminate cancer cells. Aromatase inhibitors make up the third class - an example being letrozole tablets - which block estrogen synthesis through inhibition of aromatase activity and are suitable for postmenopausal breast cancer patients. Sensitizing agents form the fourth class aimed at increasing tumor cell sensitivity towards therapeutic drugs in order to enhance treatment efficacy; an example being gold nanoparticles. Lastly, monoclonal antibody drugs such as Bevacizumab injection constitute the fifth class - these targeted therapies can precisely reach lesion sites thereby improving drug effectiveness and utilization rate [4].

2. ADCs and their mechanism of action

Currently, immunotherapy and chemotherapy are the most commonly used treatments for TNBC. However, due to the poor prognosis of TNBC patients, these two therapies have shown obvious side effects and drug resistance. In recent years, ADCs as a new type of anti-tumor drugs, have become a hot spot in the study of tumor targeted therapy. ADCs consists of three parts: monoclonal antibodies with tumor antigen specificity, cytotoxic drugs with strong killing power, and stable lytic or non-lytic linkers [1]. The mechanism of action is that ADCs uses monoclonal antibody as the carrier to recognize the surface antigen of tumor cells, and cytotoxic drugs as the “warhead”. The two can target tumor cells through linker coupling, but are almost non-toxic to normal cells, and accumulate cytotoxic drugs on the surface of tumor cells, playing a high-efficiency and low-toxicity role. ADCs bind to antigen-positive tumor cell targets, and then endocytosis into tumor cell lysosomes mediated by receptors to form early endosomes containing ADC-antigen complexes. Through a series of mechanisms, lysosomes create a low-pH environment that promotes the cleavage of ADCs and the release of toxic substances, and then cytotoxic drugs cause cell death in a variety of ways, such as DNA insertion into RNA or inhibition of microtubule polymerization. ADCs can also mediate tumor cell death by entering neighboring tumor cells through the “bystander effect” or by destroying the matrix surrounding the tumor, depending on the various effects mediated by ADCs, including CDC, ADCC, or ADCP triggering immune effector cells to release cytotoxic drugs within tumor cells, thereby inducing tumor cell death. In addition, when the targeted tumor cell surface specific antigen is a molecule involved in tumor cell growth, differentiation, proliferation, metastasis and other signal transduction, the combination of MAB with the post blocks its downstream signal transduction pathway and can also inhibit the proliferation of tumor cells [5].

3. Pharmaceutical analysis

3.1. Bevacizumab

According to research, bevacizumab has a relatively high proportion of application in first-, second-, and third-line treatments. Its treatment scope covers a variety of malignant tumors, and is clinically more effective than other drugs offer significant advantages [6]. Bevacizumab mainly exerts anti-tumor effects as a vascular endothelial growth factor (VEGF) antagonist. It specifically binds and inhibits VEGF, preventing it from binding to receptors on endothelial cells, thereby hindering the formation of new blood vessels. During tumor growth, solid tumors rely heavily on vascularization to meet their high metabolic needs, obtain nutrients and oxygen, and excrete metabolic waste products [7]. Among the angiogenic factors secreted by tumors, VEGF, especially VEGF-A, is a key factor in inducing tumor angiogenesis. VEGF activates VEGF signaling in endothelial cells by binding to VEGF receptor tyrosine kinases (VEGFR1-3), stimulating the proliferation and survival of endothelial cells while enhancing vascular permeability to meet the metabolic needs of tumor growth.

As a result, bevacizumab significantly reduces the proliferation and spread of malignant cells. Bevacizumab significantly improved overall survival, progression-free survival, and response rate when used in conjunction with other treatments as opposed to chemotherapy alone (all $P < 0.05$). However, new research has cast doubt on bevacizumab’s ability to successfully stop the growth of tumor cells; as a result, bevacizumab is mostly employed in clinical settings in conjunction with other chemotherapeutic

medications. Statistics from Zhejiang Cancer Hospital show that over 90% of bevacizumab treatments need the use of additional chemotherapy medications, as shown in the table 1.

Table 1. Bevacizumab treatment regimen [7].

Therapeutic regimen	Frequency	Constituent ratio(%)
Single-used scheme		
Bevacizumab alone	35	9.16
Co-use scheme		
In combination with platinum drugs	63	16.50
In combination with yew containing drugs	69	18.05
In combination with anthracycline-containing drugs		
In combination with antimetabolic drugs (ex. Chlorouracil)	193	50.54
In combination with other chemotherapeutic agents	16	4.18
In combination with targeted therapeutic agents	1	0.26
Total	382	100

3.2. *Gosa Tuzumab (SG)*

SG is the first ADC to be globally approved for the treatment of advanced TNBC. As the inaugural ADC targeting Trop-2, SG received accelerated approval from the U.S. Food and Drug Administration (FDA) in 2020. It was subsequently approved in 2021, for the treatment of patients with locally advanced or metastatic TNBC who had undergone at least two prior treatment regimens, including at least one for metastatic disease. For patients with locally advanced or metastatic urothelial carcinoma (UC) who have previously had treatment with platinum-based chemotherapy and a programmed death receptor and its ligand 1 (PD-1/PD-L) inhibitor. Adult patients with locally advanced or metastatic TNBC who had received at least two prior systemic therapies, including at least one for metastatic cancer, were eligible to receive SG as of June, 2022, in China. SG is comprised of a cleavable linker, an active metabolite of irinotecan called SN-38, and a monoclonal antibody called hRS7 that targets trophoblast cell surface antigen 2 [8].

Membrane glycoprotein TROP-2 is overexpressed in breast, colorectal, and urothelial carcinomas, among other epithelial cancers. It plays a role in cellular activities like migration and proliferation [8]. With a high drug-to-antibody ratio of 7.6:1, hRS7, as a carrier, maintains high stability in the bloodstream, delivering a high concentration of SN-38 to the tumor tissue. The antibody of SG specifically binds to TROP-2 on the surface of tumor cells, facilitating the internalization of SN-38 through phagocytosis [8]. SN-38, a topoisomerase inhibitor, is 20–136 times more potent than the parent compound irinotecan in xenograft tumor models, inducing DNA double-strand breaks and apoptosis [8]. Due to its pH-dependent linker, SG can directly hydrolyze and release SN-38 within the tumor microenvironment. This property allows SG to exert a direct cytotoxic effect on tumor cells with low or no expression of TROP-2, independent of antibody-mediated targeting. This phenomenon is known as the ADC bystander effect [8].

In patients with TNBC who have progressed after multiple lines of treatment, including taxanes, anthracyclines, platinum-based agents, immunotherapy, and poly ADP-ribose polymerase (PARP) inhibitors, SG continues to demonstrate significant efficacy [8]. This has shifted the paradigm for the treatment of advanced TNBC, which was predominantly reliant on single-agent chemotherapy.

3.3. *Other Medications*

3.3.1. *Datopotamab Deruxtecan (Dato-DXd, DS-1062a)*

Datopotamab deruxtecan is a therapeutic agent that incorporates a DNA topoisomerase I inhibitor, DXd. It demonstrates a high degree of specificity for TROP2, a target antigen that is abundantly expressed in

epithelial tumors. This targeted approach is designed to maximize the drug's impact on cancer cells while minimizing its effects on healthy cells [9]. Upon binding to TROP2 on the surface of tumor cells, Dato-DXd is internalized through endocytosis. Once inside the cell, it is processed through the cellular transport system and ultimately delivered to the lysosome. The acidic environment within the lysosome facilitates the release and activation of DXd [10]. Once activated, DXd initiates its therapeutic action by inducing DNA damage and apoptosis in the tumor cells, thereby contributing to tumor shrinkage and preventing its metastasis. This mechanism is pivotal to its anti-cancer efficacy [9]. Although Dato-DXd is currently in Phase III clinical trials, new medications like this may still present unforeseen risks and side effects when widely adopted, necessitating ongoing market evaluation and surveillance.

3.3.2. *Pembrolizumab*

Originally developed as a monoclonal antibody for melanoma treatment, pembrolizumab (Keytruda) has expanded its therapeutic scope. On November 9, 2022, Merck announced that pembrolizumab has received approval from the National Medical Products Administration (NMPA) of China for use in combination with chemotherapy as a neoadjuvant treatment. Following surgery, it is also used as a standalone adjuvant therapy to further enhance treatment outcomes [11]. PD-1, a protein predominantly expressed on the surface of activated immune cells such as T cells, B cells, and macrophages, plays a crucial role in immune regulation. When tumor cells express high levels of PD-L1, they can bind to PD-1 on immune cells, leading to immune evasion and shielding the tumor from immune attack. Pembrolizumab, a PD-1 immune checkpoint inhibitor (ICI), counters this by blocking the CTLA-4 or PD-1/PD-L1 pathways, thereby reactivating the immune system's ability to target and eliminate tumor cells, effectively suppressing tumor growth and metastasis [12]. It is particularly beneficial for the treatment of early-stage, high-risk TNBC patients whose tumors have been confirmed to express PD-L1 through validated testing methods.

Recent studies have demonstrated that the combination of pembrolizumab with taxane and anthracycline-based chemotherapy significantly elevates the pathological complete response rate to 44%, a substantial improvement over the 17% rate observed with chemotherapy alone. This synergistic effect is especially pronounced in patients with early-stage, high-risk TNBC, where the pCR rate has been shown to increase from 22% to 60%. Furthermore, patients achieving pCR with pembrolizumab have demonstrated significantly improved long-term survival outcomes, with a 3-year event-free survival rate reaching as high as 93% [13]. These findings underscore the potential of combining anthracycline-based chemotherapy with PD-1/PD-L1 inhibitors during the neoadjuvant phase to not only increase the likelihood of pCR in TNBC patients but also to significantly enhance their long-term prognosis.

3.4. *Drug Comparison*

In summary, these four drugs each possess unique characteristics and are targeted towards different types of cancers and patient populations. When utilizing these medications, it is imperative to select the appropriate drug based on the specific conditions of the patient and the characteristics of the disease, and to strictly adhere to medical guidance and medication protocols.

Table 2. Comparison of selected TNBC ADC drugs

Drug Name	Target	Mechanism of Action	Treatment Regimen
Bevacizumab	VEGF receptor	Inhibits tumor cell growth indirectly by suppressing the formation of new blood vessels.	Used in combination with other chemotherapy drugs.
SG (Trodelvy)	Trop-2	Undergoes receptor-mediated endocytosis, entering tumor cells where it is degraded by lysosomes to release its payload, exerting cytotoxic effects. Additionally, SG possesses antibody-dependent cellular cytotoxicity (ADCC) and signaling inhibition effects, collectively contributing to its antitumor efficacy.	Used in combination with other inhibitors.
Datopotamab Deruxtecan	Trop-2	Binds to TROP-2 on the surface of tumor cells via a humanized anti-TROP2 IgG1 monoclonal antibody. The linker hydrolyzes to release the toxin, which inhibits DNA topoisomerase I, causing DNA single-strand breaks and inducing DNA damage and tumor cell death.	Not yet widely used in clinical practice, and treatment regimens are still under discussion.
Pembrolizumab	PD-1	By binding to PD-1/PD-L1, pembrolizumab blocks the PD-1/PD-L1 signaling pathway, allowing immune cells to recognize and attack tumor cells. Additionally, pembrolizumab promotes antigen presentation, enhancing the effector function of antigen-specific cytotoxic T cells.	Combined with chemotherapy as a neoadjuvant treatment approach, and used alone as adjuvant treatment post-surgery.

Other Therapeutic Directions (Table 2). Current innovative approaches to treating TNBC can be categorized into four main areas: targeted therapy, immunotherapy, gene editing technology, and cell therapy.

For targeted therapy, identifying targets within TNBC is fundamental. PARP plays a role in DNA repair within tumor cells, and PARP inhibitors have demonstrated antitumor activity against TNBC, particularly in tumors with BRCA1 and BRCA2 mutations. This suggests their potential as targets. PARP inhibitors currently under development include Olaparib, Veliparib, and Iniparib. These drugs have shown certain antitumor activities in in vitro experiments, animal studies, and phases I and II clinical trials. However, their long-term efficacy and issues of drug resistance in the treatment of TNBC still require further investigation [14].

In the realm of immunotherapy, ICIs are commonly used. PD-1 is a crucial immunosuppressive molecule, and immune modulation targeting PD-1 holds significant importance in combating tumors, infections, autoimmune diseases, and in the context of organ transplants. The interaction between PD-1 and its ligand, PD-L1, on the surface of tumor cells can lead to the persistent activation of the PD-1 pathway, resulting in the suppression of T cell function and their inability to eliminate tumor cells, thus facilitating immune evasion by tumor cells [15]. Consequently, PD-1/PD-L1 inhibitors can be employed for the immunotherapy of TNBC.

Additionally, gene editing technology has made certain advancements. Research has found that COP1 plays a critical role in the development and spread of tumors. COP1 regulates the protein levels of the transcription factor C/ebpδ through the adapter protein Trib2, and C/ebpδ can inhibit the release

of macrophage chelators by cancer cells. In TNBC, the suppression of COP1 leads to a decrease in macrophage infiltration and an increase in sensitivity to anti-PD-1 therapy [16]. In mouse models, the knockout of COP1 significantly inhibited tumor growth and extended the survival time of mice, particularly in those treated with immune checkpoint blockade (ICB) [16].

Lastly, research on tumor stem cells provides robust evidence for cell therapy. ALDH⁺ cells, due to their high self-renewal capacity and multilineage differentiation potential, are considered key contributors to therapy resistance and recurrence in breast cancer. A research team discovered that KK-LC-1 (Kita-Kyushu Lung Cancer Antigen-1) is highly expressed in TNBC ALDH⁺ cells and promotes its ubiquitination and degradation by binding to FAT1, thereby affecting the Hippo signaling pathway. This leads to the nuclear translocation of the transcription factors YAP1 and ALDH1A1, enhancing the stemness of ALDH⁺ cells. To reverse the malignant characteristics induced by KK-LC-1 expression, researchers have identified a small molecule inhibitor named Z8 using computational methods. This compound can disrupt the binding between KK-LC-1 and FAT1, thereby reactivating the Hippo pathway. The activation of this mechanism reduces the stemness and vitality of TNBC ALDH⁺ cells, suppressing tumor growth and metastasis.

4. Conclusion

ADCs are currently a primary direction in the treatment of TNBC, primarily delivering small molecule drugs to tumor cells via VEGF receptors, Trop-2 receptors, and PD-1, thereby exerting their tumor-killing effects. In the treatment of TNBC, ADCs offer significant advantages, such as high selectivity, high efficiency, and low side effects. However, despite certain successes in the development and application of ADCs, there are still some drawbacks and challenges. The preparation process of ADCs is complex, involving multiple steps and delicate operations, which increases production costs and technical difficulty. The efficacy of ADCs is influenced by various factors, such as tumor cell heterogeneity, antigen expression levels, drug dosage, and treatment duration. This article only analyzes a portion of the drugs used for treating TNBC, which may have some limitations. With the continuous advancement of technology and in-depth clinical research, it is believed that more innovative ADCs will emerge in the future, providing more effective and safe options for tumor treatment. At the same time, research on the preparation process, stability, efficacy, and safety of ADCs will continue to deepen, promoting the application and development of ADCs in the field of tumor therapy.

Authors Contribution

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

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Progress in immune-targeted combination therapy for melanoma

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Abstract. Melanoma is a type of skin cancerous tumor that develops from melanocytes and often appears on the skin's surface, however it can also spread to other areas like the eyes and mucous membranes. Research on it has made significant progress in recent years. Immunotherapy has become an important means of treating melanoma. The use of anti-CTLA-4 and anti-PD-1/PD-L1 antibodies has achieved significant success, so some patients have achieved long-term survival. benefit. Secondly, there have been breakthroughs in targeted therapeutic drugs targeting specific molecular targets such as BRAF and MEK. It has the ability to successfully stop melanoma cells from growing and spreading. Despite significant advances in immunotherapy and targeted therapies, drug resistance remains a serious problem; metastasis of melanoma is one of the main causes of its lethality, but little is known about how melanoma cells metastasize to other organs. There are still many unanswered questions about the mechanism. This article compares the benefits of immune-targeted combination therapy with immunotherapy alone. It also immunotherapy and targeted therapy alone. It comes to the conclusion that combination therapy is the best course of action. The mechanism of action, effectiveness and drawbacks of drug resistance of the targeted therapy drug Vemurafenib were also introduced, further confirming the 1+1>2 treatment plan. Combination therapy provides new ideas for future cancer medicine, but the drug resistance of some targets needs to be solved, and subsequent research can focus on the development of new targets.

Keywords: Mitogen-activated Protein Kinase, BRAF gene, Vemurafenib, combination therapy.

1. Introduction

Melanocytes are cells that are mostly located in the choroidal layer of the eye, mucosal surfaces, meninges, basal epidermis, and hair follicles. These cells are derived from the neural crest. Melanocytes contain melanocytes that specifically produce melanin. When the skin is exposed to ultraviolet light, it acts as a response to ultraviolet-induced DNA. Skin horn cells secrete melanocyte-stimulating hormone in reaction to damage. This hormone binds to melanocortin receptor 1 on melanocytes, causing the melanocytes to generate and release melanin, which causes the skin to darken. This is the so-called sunbathing. The pigment produced by melanocytes is transferred to other cells in the skin, thereby protecting their DNA from sun damage. Ultraviolet radiation, a component of sunlight, damages the DNA of cells and causes mutations that may lead to the formation of skin cancer. Skin cancer often originates from mutations in melanocytes. Ultraviolet rays cause thymine dimers to form in the DNA of

melanocytes, disrupting their protein synthesis. Cell division is out of control, malignant tumors form, and the risk of cancer is increased. Tumor cells can be transferred to other parts of the body, including organs, through the blood circulation and lymphatic circulation, and these tumor cells can lead to further tumor formation. One of the most common human diseases to metastasize is malignant melanoma, in which a T1 submillimeter-sized primary tumor with approximately 10⁶ cell populations may have significant metastatic potential, whereas most solid cancers, with 10⁻⁹ cell populations T1 tumors that are twice the size but similar may not have metastatic potential. Due to changes in the global atmosphere, ultraviolet exposure to the skin has gradually increased, and the incidence of melanoma has also increased. Although there are currently various treatments, preventive measures are essential. People can isolate part of the skin through means such as parasols and sunscreen. UV rays reduce the risk of disease. Among all melanomas, a higher proportion of mucosal melanomas are found in the head and neck (52%), gastrointestinal tract (28%), female genitalia (9%), conjunctiva (6%), and other sites (5%) [1]. The ALM subtype of cutaneous melanoma accounts for 41%, the NM subtype accounts for 20%, the SSM subtype accounts for 19%, the LMM subtype accounts for 7%, and unknown or unclassified cases account for 13% [2].

Patients with melanoma may live longer and enjoy better quality of life thanks to new treatments, such as immunotherapy and improved surgical technique. Secondly, the use of innovative therapies such as CART cell therapy and immune checkpoint inhibitors (ICI) in melanoma treatment has promoted progress in the field of immunotherapy. These advances will not only benefit melanoma patients, but may also provide therapeutic implications for patients with other types of cancer. Traditional malignant tumors need to be cured by radiotherapy and chemotherapy, which are often accompanied by severe side effects. By studying the biological and genetic characteristics of melanoma, scientists can better understand the development mechanism of this disease and identify potential therapeutic targets. This will help develop more effective treatment strategies and reduce reliance on traditional treatments. The development of new vaccines, such as the study of the combination vaccine of mRNA-4157 (V940) and Keytruda, has demonstrated the potential to prevent relapse and improve patient survival rates. This marks an important advance in vaccine therapy in the field of cancer treatment, particularly in achieving long-term control and potential cure of the disease.

2. Immunotherapy approaches for melanoma

One of the most effective treatment strategies for melanoma is immunotherapy because this tumor is highly immunogenic. Immunotherapy works primarily on specific targets of the immune response's counter-regulatory system. One is an immune system-based vaccination strategy. Vaccine virus is one of them. It has a good oncolytic agent. It can infect cells and elicit an immunological response. The cytokines and other immune regulatory molecules it produces activate the tumor immune system. Based on viruses including adenovirus, HSV and reovirus, a number of oncolytic viruses have been created [3]. Studies have shown that the HSV can selectively proliferate in the melanoma tissues of naked mice and can induce in vitro cellular death in human melanoma cell lines [4]. Clinical experiments have demonstrated the safety of injecting oncolytic viruses intraperitoneally into individuals with melanoma. This is because the replication of oncolytic viruses not only works on tumor cells forthright, but the therapeutic agent also spreads further through the tumor tissue, making it safe and efficient. Another discovery of antibodies against specific targets is based on the immunological origin of malignancy, known as ICI. Kill cancer cells by reactivating T cells and prolong survival time.

Research shows that the main power of cancer treatment lies in drugs combination. The victory of clinical trials is inseparable from the combined use of chemotherapy, radiotherapy, ICI and targeted molecular therapy. Although the target of melanoma targeted therapy is easy to be inactivated, it is highly targeted and is an indispensable treatment method at the moment. Gene mutations in melanoma cells are mainly concentrated in the mitogen-activated protein kinase pathway (MAPK). Stimulating factors activate the downstream MAPK pathway by binding to receptors on the cell membrane. Ultimately, MAPK can phosphorylate a variety of downstream targets, transmit extracellular signals into cells, and regulate physiological and pathological processes of cells. The main genes involved in

this pathway are BRAF, NRAS and KIT. The BRAF gene mutation is one of the most common gene mutations in melanoma. The following will describe the pathogenesis and treatment ideas by introducing the mechanism of action of the main research target BRAF.

3. Activation of MAPK pathway caused by BRAF gene mutation

The BRAF gene is a proto-oncogene located on chromosome 7q34. Encodes a serine/threonine-specific kinase. Because it belongs to the subfamily of MAPKKK, it plays a key role in the mitogen-activated protein kinase pathway. By taking involvement in the cell surface stimulation of melanocortin receptors, it participates in the proliferation and differentiation process of melanocytes. Studies have found that overexpression of mutant BRAF gene products can increase the activity of downstream extracellular regulated protein kinase ERK, reduce the expression of melanoma antigens Melan-A/MART-1 and glycoprotein 100 recognized by T cells, and cause obstacles to immunotherapy. The MAPK pathway combined with the STAT3 signaling and transcriptional activator pathway can cause melanoma cells to produce a variety of immunosuppressive factors, leading to immune evasion.

The most common cause of MAPK signaling is RTK dimerization in response to GF binding. This results in the phosphorylation of certain tyrosine residues in the cell's cytoplasmic tail. The MAPK pathway's activation promotes long-term cell proliferation and prevents programmed cell death. The global MAPK pathway is made up of three layers of protein kinases that are activated in order during the MAPK pathway's cascade reaction. The majority of upstream levels consist of a group of kinases known as MAPKKKs, which, upon activation, phosphorylate and dimerize MAPKKs, which is a class of downstream dual-specificity serine/threonine kinases. MAPKKs sequentially phosphorylate the most downstream effector serine/threonine kinase group, namely MAPKs.

4. Inhibitory effect of vemurafenib on BRAF

An oral mutant BRAF inhibitor called vemurafenib prevents kinase activity, which overactivates the MAPK pathway. Experimentally, vemurafenib was demonstrated to improve survival with a 48% effectiveness rate and manageable toxicity when compared to the standard-of-care drug dacarbazine. Mutation testing for metastatic melanoma has become important with the invention of vemurafenib. The prospective test is highly suitable for all patients with thick melanomas or local lymph node involvement after resection, and can significantly reduce the risk for systemic treatment of metastatic disease in the future. In melanoma, V600E and its codon-associated mutations activate the MAPK pathway. Vemurafenib is a small molecule kinase inhibitor which selectively attacks activated BRAF V600E and it is utilized to suppress the mutant serine-threonine kinase BRAF. By engaging with the ATP binding site of BRAF V600E kinase specifically, it suppresses its activity. Vemurafenib's biochemical affinity for mutant BRAF results in a strong suppression of ERK phosphorylation and BRAF mutant cell line growth. Vemurafenib reduced tumor growth in cells with the BRAF V600E mutation in animal models. Clinical trials using vemurafenib in patients with metastatic melanoma that was incurable and had BRAF V600E mutations showed impressive response rates from the range of 50% to 80%. The median progression-free survival time was increased in the vemurafenib group, as well as the median overall survival time. About 25% of individuals receiving vemurafenib developed corneal acanthoma-type cutaneous squamous cell carcinoma with little invasive potential and no metastases. BRAF mutations are also found in other solid cancers such as papillary thyroid cancer. Vemurafenib has also been tested in these entities with good results.

5. Disadvantages of vemurafenib – drug resistance

One significant issue is that most patients become resistant to vemurafenib within a few months of treatment, and there are various ways in which resistance can arise. Its epigenetic mechanisms include DNA methylation, non-coding RNA, histone modifying enzymes and histone modifications. Furthermore, a key factor in resistance to BRAFi is the tumor microenvironment. Drug resistance has been linked to intratumoral fibroblasts and macrophages, according to recent research. Cancer-associated fibroblasts (CAFs) is different of normal fibroblasts in that CAFs upregulate the expression

of vimentin, fibroblast activation protein-1 (FAP1), α -smooth muscle actin (SMA), and PDGFR and TGF β signaling [5]. According to reports, CAFs permit BRAFi's therapeutic escape.

Melanoma cells have an aggressive character in the presence of CAFs. Following BRAFi treatment, melanoma cells continue to exhibit high levels of mTOR activity signaling, which encourages the synthesis of proteins, cell division, and the uptake of nutrients in the surrounding environment [6]. Additionally, they react to cytokines and growth factors generated by CAFs, including as VEGF and TGF- β , which encourage cell growth and survival [5]. Vemurafenib directly stimulates fibroblasts to release hepatocyte growth factor (HGF), triggers the signaling pathways PI3K/AKT and MAPK/ERK, and reduces the expression of genes that promote apoptosis [7]. Interestingly, senescent fibroblasts associated with senescent melanoma secrete sFRP2 and are more invasive, which suppresses the production of MITF and apurinic endonuclease (APE1), and makes the cells resistant to BRAFi [8]. Additionally, CAFs release components of the extracellular matrix (ECM), and resistance to BRAFi is promoted by integrin signaling induced by the ECM. In terms of interaction, TGF- β is released by BRAFi-resistant melanoma cells, which encourages CAF formation and raises the expression of ECM components, eventually resulting in the emergence of BRAFi resistance [9].

6. Combination therapy is considered the best option

According to clinical trials, patients with braf-mutant melanoma now get standard targeted therapy consisting of vemurafenib and cobimetinib, which has been shown to have a greater response rate, an improved overall survival of around two years, and reduced skin damage. Combined inhibition of PI3K/AKT and MAPK can enhance BRAF mutant melanoma's perception to BRAFi or MEKi, and the two have a synergistic effect in inducing apoptosis [10]. As uveal melanoma with activating Q209 L/P mutations was studied, the pan-PI3K inhibitor GSK2126458 and the MEKi GSK1120212 together significantly increased apoptosis as compared to when each pathway was inhibited alone [11]. By combining PI3K/AKT inhibitors, the effectiveness of MAPK inhibitors can be increased while also potentially overcoming resistance. According to one study, drug-resistant melanoma did not exhibit decreased phosphorylation of AKT and MAPK downstream target S6, indicating that AKT pathway hyperphosphorylation may play a role in drug resistance. This is corroborated by the fact that BRAFi, MEKi, and an AKT inhibitor restored melanoma cell resistance to vemurafenib [12]. Similarly, dafinib or trametinib plus the PI3K inhibitor GSK2118136 will postpone the onset of resistance.

The clinical effectiveness of BRAFi or MEKi in combination with PI3K/AKT inhibitors has been underwhelming, despite strong preclinical findings. Only few melanomas reacted in a phase 1 clinical trial evaluating the safety of trametinib in conjunction with the AKT inhibitor afresertib.

ICIs include PD-1 and CTLA-4 receptor inhibitors (i.e., pembrolizumab, nivolumab, and ipilimumab), which exhibit longer-lasting effects than BRAFi but lower response rates. The combination of BRAFi and MEKi with ICIs may be able to overcome the drawbacks of each type and produce responses that are more persistent, according to preclinical and translational studies [13]. According to mechanistic research, BRAF inhibitor treatment, either by alone or in combination with MEK inhibitors, has been associated with increased T cell toxicity, increased CD8⁺ T cell infiltration, and decreased production of immunosuppressive cytokines, such as IL-6 and IL-8. Immunotherapy's potential in conjunction with BRAF/MEK inhibitors [14].

Clinical trials are currently being conducted on dual-target MAPK inhibitors in conjunction with ICIs for the treatment of melanoma. COMBI-i had the most effective rate, at 78%, according to the results of Keynote-022, IMspire 150, and COMBI-i. For the dual-target group and the three-drug group, there was no statistically significant difference in the objective effective rates across the three experiments. According to the data, the dual target's efficiency has achieved its peak. For the treatment of melanoma, dual-target MAPK inhibitors are presently undergoing clinical trials in combination with ICIs. Based on Keynote-022, IMspire 150, and COMBI-i findings, COMBI-i had the highest effective rate, at 78%. The objective effective rates for the three experiments did not differ statistically significantly for the dual-target group or the three-drug group. The data indicates that the efficiency of the dual target has peaked [15].

7. Conclusion

This article explores the pathogenesis of melanoma and compares various treatment methods through statistical analysis of clinical data of melanoma patients. Research results show that the occurrence of melanoma is related to gene mutations such as BRAF, NRAS and KIT. This article mainly explores the impact of BRAF gene mutations on the MAPK pathway to cause tumors, as well as the pros and cons of vemurafenib targeted treatment of melanoma. Numerous studies have found that trametinib plus targeting PD-1, PD-L1, or CTLA-4 may be a more effective anti-tumor combination therapy than either of the two alone. This is because blocking the MAPK pathway and immune checkpoint signaling at the same time may be beneficial. This means that combined immune and targeted therapy is by far the best solution. However, we still have a long way to go to deal with the inevitable drawbacks of tumor drug resistance and the easy failure of targets. It is hoped that future research can focus on the development of new targets, which will be conducive to the development of innovative drugs and personalized medicine. It may also reduce drug side effects and drug resistance and other aspects of problems. It will promote basic medical research and brings new hope for disease treatment.

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Mechanism of Astragalus polysaccharides (APS) improving myelosuppression and exerting anti-tumor effects during radiotherapy and chemotherapy

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Abstract. Chemoradiotherapy, as a routine method of anti-tumor treatment, is accompanied by severe adverse reactions such as myelosuppression. It not only hinders the treatment process but also reduces the quality of life of patients. Therefore, effective prevention and treatment of myelosuppression during radiotherapy and chemotherapy is an extremely important issue that needs to be addressed in clinical practice. The commonly used method for preventing and treating myelosuppression during radiotherapy and chemotherapy is medication intervention. Human granulocyte colony stimulating factor(Human G-CSF) is the preferred drug, while modern Chinese medicine preparations are used as adjunctive therapy. There are still certain limitations in the clinical use of Human G-CSF, so research on the unique role of modern Chinese medicine in this regard has gradually received attention. By consulting literature, we took Astragalus polysaccharide (APS), a traditional Chinese medicine extract that has been proven to have relevant preventive and curative effects in existing studies, as an entry point to explore the mechanisms involved in the modulation of the signaling pathway by APS to play a role in improving the myelosuppressive and anti-tumour effects. This study aims to explore the mechanism by which APS participates in regulating signaling pathways to improve myelosuppression and anti-tumor effects. Some of its mechanisms are closely related to traditional Chinese medicine theory. This article will briefly discuss them in order to provide some inspiration for expanding drug selection in clinical practice and further conducting related research.

Keywords: APS, myelosuppression with radiotherapy and chemotherapy, traditional Chinese medicine, pharmacological effects and mechanisms

1. Introduction

Chemoradiotherapy induced myelosuppression refers to one of the most frequent adverse reactions during anti-tumor treatment. Chemoradiotherapy inhibits bone marrow hematopoietic activity, decreases the number of blood cells, reduces their resistance and self repair ability, and in severe cases, may cause infections, anemia, and so on. It becoming the main obstacle to promoting treatment and improving prognosis. The commonly used drug for treating myelosuppression in clinical practice is polyethylene glycol recombinant human granulocyte colony stimulating factor (PEG-rhG-CSF). Its main action is to promote the growth of peripheral blood cells, but it has more adverse reactions, most

commonly occurring in the skeletal and digestive systems. Moreover, this drug is expensive and only effective for a short period of time, which makes it difficult to disseminate [1,2]. Existing studies have shown that modern Chinese medicine has the advantages of multi-targets, multi-links, wide sources and high safety [1,3]. It has been widely used in the treatment of myelosuppression after radiotherapy and chemotherapy. From the perspective of traditional Chinese medicine theory, radiotherapy and chemotherapy are classified under the category of heat toxin [4]. Heat is injurious to fluids, and fluids and blood are homologous to each other, thus chemoradiotherapy can cause depletion of fluids and blood in the body. The concept of myelosuppression caused by radiotherapy and chemotherapy in Western medicine is highly consistent with the traditional Chinese medicine belief that heat toxin tend to deplete the body's fluid and blood. In terms of treatment principles and methods in traditional Chinese medicine, as the saying goes, 'deficiency is replenished', 'loss is gained', and 'supplementing Qi and generating blood'. Therefore, in clinical practice, drugs with sweet taste, warm nature, and the effect of generating fluids and nourishing blood are often used for tonifying [5] to improve the body's immune system and promote hematopoietic function. Research has shown that *Astragalus membranaceus* has the highest frequency of occurrence in the prevention and treatment of myelosuppression after radiotherapy and chemotherapy among Chinese medicinal herbs for tonifying deficiency [3,4].

APS is an effective active ingredient extracted from *Astragalus membranaceus* that has a Qi tonifying effect. Modern research suggests that it has pharmacological effects such as promoting hematopoiesis, enhancing immune function, and protecting bone marrow [6,7]. This article will start from the theory of traditional Chinese medicine and briefly introduce the effect of APS on p53-p21 to improve myelosuppression during radiotherapy and chemotherapy, as well as Wnt/ β -catenin exerts anti-tumor effects through two signaling pathways is expected to provide new insights for the further application of modern traditional Chinese medicine in the clinical treatment of myelosuppression after tumor radiotherapy and chemotherapy.

2. Mechanism of action of APS

2.1. Analysis of active ingredients

APS was isolated and extracted from *Astragalus membranaceus* from a class of biomacromolecular compounds consisting of glucan (Glu) (including water-soluble glucan and water-insoluble glucan), heteropolysaccharides (mostly acidic heteropolysaccharides, including glucose, arabinose, rhamnose, galactose, and glycoalkaloid acid, etc.), and some proteins that may be contained as well as trace elements, as polysaccharides contain many branched chains and a large number of hydroxyl groups. Their chemical structures are extremely complex and difficult to determine [8,9]. Some studies have shown that APS is mainly composed of glucose, galactose, arabinose and other monosaccharides linked by β -1,4-glycosidic bonds to form a linear polysaccharide structure [10]. Different *Astragalus* species, origins, and preparation methods may lead to differences in the chemical composition of APS, which may affect its biological activity. APS mainly has immunomodulatory, anti-tumour, hematopoietic and bone marrow protection pharmacological effects.

2.2. p53-p21

p53 is an important transcription factor involved in the regulation of apoptosis. Tang Shihuan et al. [11] found that p53-p21 is involved in the process of stem cell senescence, and once this pathway is activated, the self-renewal ability of stem cells is reduced, thus inducing cell senescence or even apoptosis. APS may be able to reduce the damage to hematopoietic stem cells (HSCs), enhance their self-repairing ability, and allow more cells to participate in the cell cycle by reducing the expression of p53 and inhibiting the aberrant activation of p53-p21 signaling pathway associated with stem cell senescence, in order to slow down the senescence of HSCs. see Figure 1. Modern Chinese medicine attributes chemoradiotherapy to the category of heat toxin, and a high level of heat can easily deplete the body's fluid and blood, resulting in a relative deficiency of Qi and blood. *Astragalus* is a sacred medicine for tonifying Qi, which has the efficacy of generating fluids and nourishing blood. Chinese medicine is

based on the theoretical foundation of ‘Qi and blood mutualisation’ and ‘Qi can generate blood’, and has always had the principle of ‘tonifying Qi and generating blood’, which is considered to be the most effective way of treating the problem of blood circulation. It is believed that by using a large number of Qi tonic products, the tangible yin and blood can be generated. APS can reduce the expression of pro-apoptotic gene/protein p53 and inhibit the activation of this pathway by acting on the p53-p21 signaling pathway, thus enhancing the self-repairing ability of HSCs, slowing down the aging of HSCs, and inhibiting apoptosis, which is consistent with the traditional Chinese medicine theory of ‘when positive qi exists in the internal body, evil can not be interfered with’. This statement is from the classic Chinese medical text ‘The Inner Canon of Huangdi’, which means that if the body has sufficient positive Qi, it will not be easily attacked by external evils to cause disease. Many studies have suggested that positive Qi may be related to the immune system. Modern pharmacological studies have shown that APS does have the effects of regulating the immune system, delaying the aging of HSCs, inhibiting apoptosis of HSCs, and treating chemotherapeutic myelosuppression [1,7].

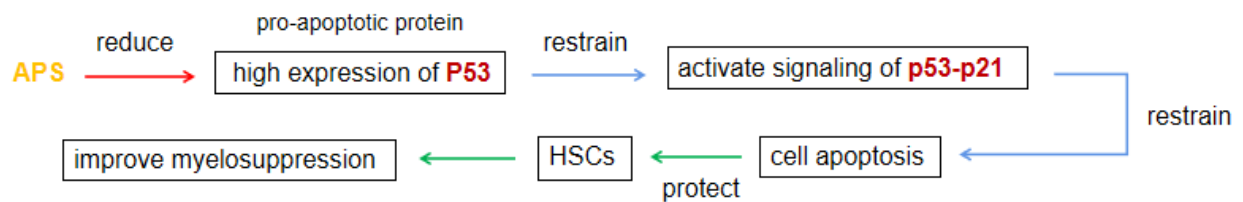


Figure 1. Mechanism of improved myelosuppression by APS.

2.3. Wnt/ β -catenin

The classical Wnt/ β -catenin signaling pathway (β -catenin-dependent) is mainly involved in the regulation of the cell cycle and a variety of biological functions, and is particularly closely related to cancer development [12]. It has been shown that lowering the expression of Wnt1 ligand can inhibit the aberrant activation of the Wnt/ β -catenin signaling pathway, thereby slowing down the proliferation of cancer cells in mouse models of breast cancer. On the contrary, if Wnt1 is overexpressed, it promotes tumour growth [13]. A study by Tan Wenchao et al. [14] also found that APS significantly down-regulated the expression of Wnt1 and β -catenin proteins in a dose/concentration-dependent manner, which in turn inhibited the proliferation of breast cancer cells, and to a certain extent reduced the probability of tumours developing distant metastasis and/or invasion. Experiments showed that the 4.0 mg/mL APS administration group was able to further reduce the expression of Wnt1 and β -catenin protein compared with the 2.0 mg/mL APS administration group. See Figure 2, the mechanism by which APS acts on the classical Wnt/ β -catenin signaling pathway to exert anti-tumour proliferation and differentiation effects (Figure 3).

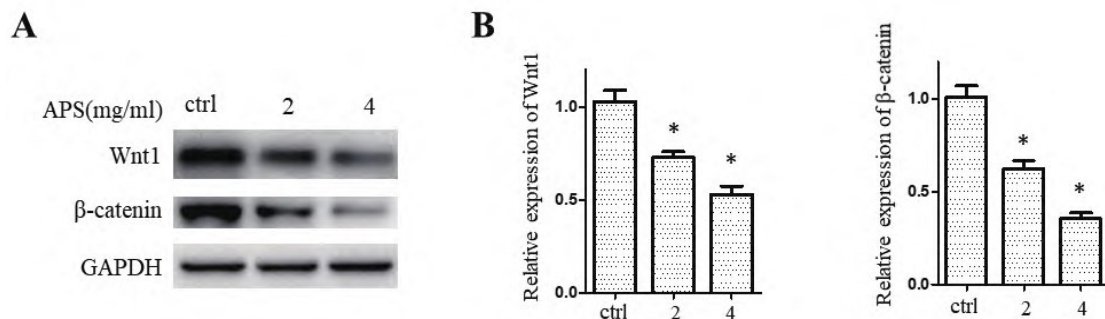


Figure 2. Expression of Wnt1 and β -catenin Sourced from reference [14].

Note: A. Western blot detection of Wnt1 and β -catenin protein expression levels;
Quantitative analysis of Wnt1 and β -catenin protein expression; *P<0.05.

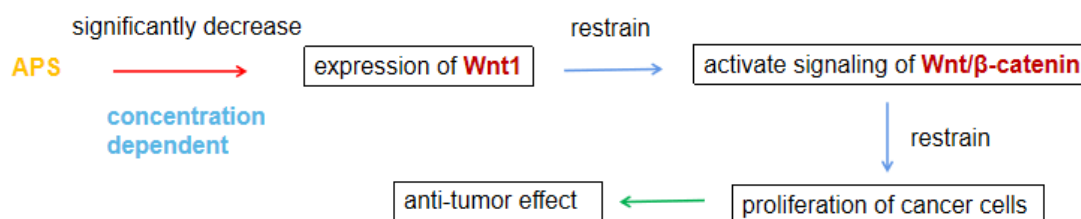


Figure 3. Diagram of APS anti-tumour mechanism.

3. Conclusion

This paper discusses the two signaling pathways that play a role in the treatment of cancer and myelosuppression, an adverse effect that occurs in the course of treatment, and finds that APS, the main bioactive ingredient extracted from the traditional Chinese medicine Astragalus, can play a vital role in reducing the expression of key genes, p53 and the ligand, Wnt1, and inhibiting the aberrant activation of the relevant pathways. At present, in the context of the urgent need to solve the problem of myelosuppression in chemoradiotherapy, it is particularly necessary to return to the classical theories of traditional Chinese medicine for inspiration, and then go deeper into the exploration of modern traditional Chinese medicine. However, this paper only focuses on two of the many complex pathways related to tumours, and there are still limitations and deficiencies in the further analysis of related pathways. At present, APS has been widely studied in clinical research, and many studies have been devoted to continue to advance its pharmacological mechanism in the face of many technical and methodological difficulties. In the future, research can pay more attention to the development of the combination of Chinese and Western medicines, in order to better complement each other's strengths and weaknesses, and to point out a new way out for clinical treatment means and medication guidance.

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Therapeutic effect and mechanism of action of geniposide in rheumatoid arthritis

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Abstract. Rheumatoid arthritis is a systemic autoimmune disease with persistent, symmetric and polyarthritis as the main clinical manifestations, which can improve the risk of cardiovascular disease and lymphoma. Untimely or inadequate treatment will result in a high rate of disability. In the context of the development of Chinese medicine modernisation, in order to explore the therapeutic effects and mechanisms of the diseases, researchers have extracted the active ingredients of Chinese herbal medicine for experiments. Geniposide through modern research found that it has anti-inflammatory analgesic and other effects, and has therapeutic effect on rheumatoid arthritis. But at present for geniposide's action mechanism and the pathogenesis of rheumatoid arthritis are not completely clear. This paper analyses the geniposide anti-inflammatory effect and analgesic effect aspect research, finds that geniposide can have a therapeutic effect on rheumatoid arthritis through anti-inflammatory analgesic effect. At present, the mechanism of action of geniposide against rheumatoid arthritis has not been fully defined, and geniposide has hepatotoxicity and many uncertainties in high concentrations, so it is of great significance to explore its action mechanism and provide theoretical reference for the new drugs' development to treat rheumatoid arthritis. The future research can pay more attention to explore the geniposide anti-inflammatory effect on the cell signalling pathway mainly embodied in a specific target, as well as geniposide development as a new drug possible direction.

Keywords: Geniposide, Rheumatoid arthritis, anti-inflammatory effect, analgesic effect.

1. Introduction

Synovial inflammation is the biological foundation of rheumatoid arthritis (RA), a chronic systemic autoimmune disease defined by the infiltration and erosion of inflammatory cells [1]. As RA is associated with a wide range of factors, including a series of immune responses, antigens, macrophages, and cytokines, it causes degradation of cartilage and bone in patients, ultimately leading to joint deformity and dysfunction [1]. Although its pathogenesis is not yet clear, current studies have shown that the interaction between genetics and environment is closely related to the initiation of RA [2]. At this time, both T and B cells are activated, and T cells activate B cells to produce autoantibodies like rheumatoid factor (RF) at this point. Activated T cells are recruited to the synovial membrane to activate macrophages and stimulate them to produce a substantial amount of inflammatory factors, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, and IL-1b, which make Fibroblast-like synoviocytes (FLS) in the synovial membrane excessive proliferation and causes osteoarticular injuries [2].

Chinese medicine gardeniae-fructus is the dried mature fruit of *Gardenia jasminoides* Ellis in the family of Rubiaceae, which has the efficacy of clearing heat and relieving fidgetness, removing pathogenic heat from the blood and toxic material from the body, and relieving swelling and pain for external use [3]. As shown in Figure 1, geniposide (GE), is an iridoid glycoside compound, with the development of modern pharmacology, research shows that geniposide has hepatoprotective, choleretic, anti-inflammatory, analgesic, hypoglycemic, neuroprotective and other pharmacological effects [3]. It has been found that geniposide have the effect of improving RA through research [1].

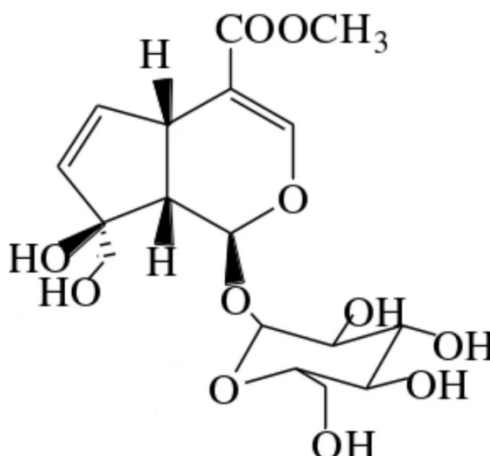


Figure 1. GE structural formula [3].

Although the existing research have proved that GE has therapeutic efficacy on RA, the mechanism of action of GE against RA is still not perfect, some molecular mechanisms have not been clarified, and a complete summary of the mechanism of action of GE against RA has not appeared. By summarizing the therapeutic effects of GE on RA under current research, the author found that GE mainly treats RA through anti-inflammatory and analgesic effects. Therefore, this paper will summarize and deeply explore the mechanisms of GE's anti-inflammatory and analgesic action.

In terms of scientific research, scientists have found that naturally occurring chemicals hold good promise in the development of new medicines, and as a result, naturally occurring chemicals have become an important source or precursor for the treatment of different diseases. This study can provide theoretical support for the development of new drugs for the treatment of RA using GE as a biopharmaceutical. This study allows researchers of GE to refine the mechanism of action of their anti-inflammatory and analgesic effects.

2. Anti-inflammatory effects of gardenia geniposide

2.1. Effects of geniposide on T cells

CD4⁺T cells can be categorized into four subpopulations like pro-inflammatory Th1 cells, anti-inflammatory Th2 cells, Th17 cells and regulatory T (Treg) cells [4]. It has been found that Th17 cells are a type of CD4⁺T cells characterized by the secretion of IL-17, which induces many pro-inflammatory cytokines, chemokines also produce other inflammatory cytokines involved, which in turn leads to the clinical symptoms of RA [4]. Additionally, Tregs can inhibit the immune response in order to exert anti-inflammatory effects, mainly through the expression of Forkhead box protein 3 (Foxp3) [4].

In the treatment of rats with adjuvant arthritis, GE was found to decrease the influence of Th17 cytokines and increase the expression of Treg cytokines by raising the number of Treg cells, thus GE exerts anti inflammatory action by regulating T cells [4]. Studies have shown that ulcerative colitis (UC) in animals can be treated by GE. In the treatment of UC mice with GE, it was found that the levels of IL-1 β and TNF- α in the colon and serum of mice with UC were reduced and the expression of FOXP3, which can inhibit the immune response to alleviate inflammation, was found to be increased in the colon,

so it was found that GE could alleviate the UC intestinal inflammation and barrier damage through the increase of the Treg cell differentiation of splenocytes [5].

2.2. *Geniposide ameliorates inflammatory cytokines*

When the body has an inflammatory reaction, it will induce the release of cellular inflammatory cytokines or chemokines, like TNF- α , IL-8, IL-6, IL-1 β , etc., and the production of much inflammatory cytokines will induce the inflammatory reaction; Meanwhile the release of certain factors (such as IL-8) accelerates endothelial cell damage, causing stagnation of microcirculatory blood flow, resulting in organ function damage and even leading to tissue necrosis [6].

TNF- α is involved in normal inflammatory and immune responses. The massive expression of TNF- α at the onset of RA leads to a series of clinical symptoms and destruction of local joint tissues, and is due to the fact that overexpressed TNF- α promotes the massive expression of other inflammatory factors (e.g., IL-6, IL-17, etc.) [1]. It was found that GE can effectively inhibit peripheral blood inflammatory factors and significantly reduce the serum levels of TNF- α and IL-6 in rats with RA, and GE plays an anti-inflammatory role by improving inflammatory factors [1]. Asthma is an inflammatory process characterized and associated with Th2 cytokines [7]. The Th2 response is associated with a range of inflammatory cytokines and chemokines, particularly IL-4, IL-5 and IL-13 [7]. Yanhong Deng et al. found that GE reduced the increase of IL-4, IL-13 and IL-5 and the expression of vascular cell adhesion molecule-1 (VCAM-1) in mice with ovalbumin-induced allergic airway inflammation treated with GE [7].

2.2.1. *The NF- κ B signalling pathway*

NF- κ B is a transcriptional factor that can regulate inflammatory genes and has a crucial role in inflammation [8]. When the organism is in an inflammatory state, NF- κ B is activated and NF- κ B p65 is transferred to the nucleus to regulate the expression of inflammatory genes [8].

Bin Yu et al. found that NF- κ B is activated in a mouse model of *Staphylococcus aureus*-induced pneumonia [8]. By establishing an animal model and designing a control group, a *Staphylococcus aureus* group, and GE group, the NF- κ B expression was detected by western blotting, and found that GE's protective effect against *Staphylococcus aureus*-induced pneumonia was attributed to its ability to weaken NF- κ B activation and inhibit inflammatory cytokines expression [8]. A20, a negative regulator of inflammation that plays a key role in activating NF- κ B, mainly through the TNF receptor and toll-like receptors (TLR) pathway, has been shown to exert neuroprotective effects through anti-inflammatory actions. [9]. Qian Sun et al. found that GE could improve the ischemia-induced upregulation of TRAF-6 and p-NF- κ B and increase the expression of A20, thus exerting anti-inflammatory effects through the NF- κ B signaling pathway [9].

2.2.2. *MAPK signaling pathway*

Mitogen-activated protein kinase (MAPK), a member of intracellular serine-threonine protein kinase superfamily members, is a central node of several signal transduction pathways [10]. Extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (jnk) together form the MAPKs pathway [10].

MAPK/ERK is a protein kinase, and this signaling pathway has been implicated in diseases such as tumors, inflammation, and diabetes [6]. During the experiments exploring the anti-inflammatory effects of brain microvascular endothelial cells, it was found that GE could activate the P2Y₁₄ receptor and thus inhibit the expression of the downstream ERK1/2 signaling pathway, which down-regulated the overexpression of pro-inflammatory cytokines (IL-8, MCP-1, and IL-1 β) to play an anti-inflammatory role [11].

Activation of the p38-MAPK signal pathway increases the production of downstream inflammatory cytokines, and blocking this signaling pathway reduces cytokine production, prevents chondrocyte apoptosis, and has anti-inflammatory effects [10]. Yuan Chen et al. [10] found that GE could attenuate

the phosphorylation of p38 and synergize with p38 MAPKs inhibitors to inhibit the p38-MAPK signaling pathway and achieve anti-inflammatory effects.

The anti-inflammatory effect of GE and its mechanism of are described above, from the outside to the inside to the deep. GE plays a role in alleviating inflammation through regulation of T cells and amelioration of inflammatory factors. The improvement of inflammatory factors is mainly achieved by affecting the biological molecules in the signaling pathway to achieve anti-inflammatory effects, such as affecting the NF- κ B to cause changes in the signaling pathway to achieve anti-inflammatory effects, affecting the P2Y₁₄ receptor to inhibit the expression of ERK1/2 signaling pathway to achieve anti-inflammatory effects, as well as affecting the phosphorylation of p38 to inhibit the role of the p38-MAPK signaling pathway to achieve the effect of anti-inflammatory effects.

3. The analgesic effect of geniposide

It was found that geniposide dose-dependently reduced acetic acid induced twisting in mice and increased the pain threshold of mice in the hot-plate experiment, and the analgesic effect of GE could be partially inhibited by naloxone with L-Arg, so the analgesic mechanism of GE is related to the synthesis and release of opioid receptors and NO [12]. In addition, GE can alleviate the mechanical nociceptive response in diabetic rats by inhibiting the activation of Spinal dorsal horn microglia, decreasing the level of cytokines in the spinal cord, and lowering blood glucose [12].

The EGFR/PI3K/AKT signaling pathway exerts important effects on proliferation, growth, cell migration, metabolism and survival [13]. In the sciatic nerve chronic crush injury model (CCI) experiment, it was found that GE relieved neuropathic pain, while the addition of an EGFR agonist reduced the analgesic effect of GE [13]. Dan-Dan Zhang et al. found that GE reduced EGFR/PI3K/AKT pathway activity to improve CCI symptoms, attenuate mechanical pain threshold, and relieve pain after in-depth analysis [13].

4. Current status of geniposide in RA

It has been shown through many studies [7,13] that GE has good anti-inflammatory and analgesic effects to relieve inflammation and pain. The mechanism of action of GE anti-inflammatory concerns the p38-MAPK signaling pathway [14]. Research has shown [14] that the p38-MAPK signaling pathway is a popular target for anti-inflammatory drug design, and that it is by inhibiting the p38-MAPK-induced stress response that will block the production of pro-inflammatory cytokines thereby ameliorating RA. However, the regulatory and feedback mechanisms involved in p38-MAPK have led to different intracellular pathways crosstalking each other, interacting, inhibiting or regulating p38-MAPK through phosphorylation or dephosphorylation thereby limiting its clinical potential and leading to the discontinuation of many clinical trials [14]. The anti-inflammatory effects of MSK1/MSK2 may also be inhibited, for example, by inhibiting p38-MAPK [14]. GE has been found to have a variety of pharmacologic effects through modern research, but clinical trials have found GE to be hepatotoxic [15]. In an experiment, the hepatotoxicity of geniposide in SD rats and Wistar rats and ICR mice was found that GE could cause toxic responses at high doses [15]. Wang Bo et al. experimented with different concentration grades of aqueous extracts, alcoholic extracts, and GE of gardeniae-fructus, and confirmed that too much concentration of gardeniae-fructus can cause obvious hepatotoxic reactions [15].

The anti-inflammatory effects of GE are broad, involving many different cellular pathways, but there are no relevant clinical studies to demonstrate whether GE also produces a lack of anti-RA efficacy resulting from different intracellular pathways crosstalking each other. Therefore, in the future development, the research of GE needs to consider not only its toxic response, but also whether it has better efficacy within the dose range, due to the fact that GE has too many unpredictable results in the treatment of RA, with a certain degree of risk leading to the fact that at present there is no new drug research and development with GE as the main drug.

5. Conclusion

The anti-inflammatory and analgesic effects of the Chinese medicine gardeniae-fructus extract GE are remarkable, with multi-system and multi-target therapeutic effects on RA. GE has a broad spectrum of anti-inflammatory effects involving many intracellular signaling pathways, indicating a broad spectrum of targets that bind to GE as well as a broad spectrum of anti-inflammatory therapeutic effects upon binding. Meanwhile, the analgesic effect of GE can reduce the mechanical pain threshold and relieve the pain. Although the anti-inflammatory effect of GE has been widely recognized, however, the molecular mechanism of its regulation of inflammatory response for the treatment of RA has not been completely clarified, and GE has hepatotoxicity and uncertainty in high concentration, so it is meaningful to explore its mechanism of action and provide theoretical references for new drugs development for the treatment of rheumatoid arthritis with GE as the raw drug. This article focuses mostly on the mechanism of action of the therapeutic effects of GE on RA, and its modern applications have not been carefully expanded and explored. The author believes that future studies could focus more on exploring the effects of GE's anti-inflammatory effects on cell signaling pathways mainly on a specific target, as well as on the direction of the possibility of developing GE as a new drug.

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