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The Role of Ferroptosis in Major Depressive Disorder

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Abstract

Objective: *To study the relationship between ferroptosis genes and Major Depressive Disorder (MDD).*

Methods*: The GEO database was utilized to obtain chip data and clinical information from three datasets, namely GSE98793, GSE39653, and GSE52790. To identify differentially expressed ferroptosis genes, an analysis was conducted on genes that showed differential expression between individuals with Major Depressive Disorder (MDD) and healthy controls. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes Pathway (KEGG) enrichment analyses were performed on the differentially expressed ferroptosis genes. Screening of these genes was carried out using the Lasso Regression and Support Vector Machine (SVM) methods. A diagnostic model for depression was established using logistic regression with the screened genes. The model was validated using Receiver Operating Characteristic (ROC) Curve analysis. Furthermore, the Gene Set Enrichment Analysis (GSEA) method was applied to analyze the genes included in the model. The immune infiltration of MDD and healthy controls was assessed using the Cibersort method, along with an analysis of the correlation between immune cells and ferroptosis genes. The ferroptosis gene interaction network was examined using Cytoscape software. Additionally, the DGIdb website was consulted to predict potentially effective therapeutic drugs for ferroptosis genes closely associated with MDD.*

Results*: A total of 18 genes involved in ferroptosis were identified through differential expression analysis comparing individuals with major depressive disorder (MDD) and healthy controls. Enrichment analysis, including GO and KEGG, revealed concentrated pathways related to oxidative stress response, hydrogen peroxide response, reactive oxygen species response, FoxO signaling pathway, fluid shear stress, and atherosclerosis. To identify key genes related to ferroptosis in MDD, Lasso regression and SVM techniques were employed, resulting in the selection of 10 genes. The depression diagnostic model, which utilized these 10 genes, achieved an area under the curve (AUC) of 0.773. The Gene Set Enrichment Analysis (GSEA) focusing on individual genes demonstrated that Parkinson's disease, Huntington's disease, and oxidative phosphorylation pathways were highly enriched. The analysis of immune infiltration further revealed significant differences in the resting NK cells and M2 macrophages between individuals with MDD and control subjects. Specifically, PHF21A was found to be closely associated with resting NK cells in MDD, whereas METTL14 and MAPK14 were closely related to M2 macrophages. The RNA interactions network of ferroptosis genes indicated a complex regulatory process, providing valuable insights for future research in this field. In terms of potential therapeutic options, ALOX15B, MAPK14, PRKAA1, and MICU1 among the 10 ferroptosis-related genes were found to have potential for effective therapeutic drugs.*

Conclusion: *Among all ferroptosis genes, ALOX15B, MAPK14, PRKAA1, PHF21A, MICU1, KLF2, METTL14, TP63, PARK7, PARP4 are closely related to MDD and have diagnostic value. Among them, ALOX15B, MAPK14, PRKAA1 and MICU1 may have potential effective therapeutic drugs.*

Keywords: Major Depressive Disorder (MDD), Ferroptosis Genes, ALOX15B, MAPK14, PRKAA1, MICU1.

Abbreviations: MDD, Major Depressive Disorder; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis.

1. Introduction

Depression is one of the most prevalent mental disorders worldwide, affecting millions of people across different age groups and socioeconomic backgrounds. Known as a leading cause of disability, depression has profound impacts on individuals' quality of life and poses a significant burden on society as a whole [1]. The heterogeneity in symptom presentation, treatment response, and underlying pathophysiology has made the diagnosis and management of depression challenging.

In recent years, there has been a growing interest in identifying and understanding the biological markers associated with depression. Biomarkers are measurable indicators that can objectively reflect the presence, severity, or progression of a disease. In the context of depression, the identification of specific biological markers holds great promise in improving diagnostic accuracy, aiding in the development of personalized treatment plans, and predicting treatment outcomes.

Ferroptosis is a iron dependent cell death, which is different from apoptosis, cell necrosis and autophagy. The main mechanism of ferroptosis is that under the action of iron bivalent or ester oxygenase, the highly expressed unsaturated fatty acids on the cell membrane are catalyzed to undergo lipid peroxidation, thus inducing cell death [2]. Ferroptosis is first proposed in 2012 [3]. Many studies have confirmed that Ferroptosis is an important mechanism of nerve cell damage [4-9]. Ferroptosis is also closely related to oxidative stress and inflammation. Both oxidative stress and inflammatory response are the main pathological mechanisms of depression [10, 11]. Therefore, the role of ferroptosis in depression has received attention. The purpose of this study was to investigate the relationship between ferroptosis related genes and depression by bioinformatics analysis. By elucidating the biological underpinnings of depression, we can enhance our understanding of the disorder and develop more targeted and personalized approaches to prevention, diagnosis, and treatment. Ultimately, the identification and validation of reliable depression biomarkers can lead to improved patient outcomes and a reduction in the overall societal burden of this debilitating mental health condition.

2. Methods

2.1. Obtain Data

The data analyzed came from the following 3 datasets in the GEO database. GSE39653, which included full blood samples from 21 people with MDD and 24 healthy controls. Whole transcriptome sequencing was performed using the GPL10558 platform (Illumina HumanHT-12 V4.0 expression beadchip). GSE52790, which included full blood samples from 10 people with MDD and 12 healthy controls. Whole transcriptome sequencing was performed using the GPL17976 platform ([hGlue_3_0_v1] Affymetrix Human hGlue_3_0_v1 Array). GSE98793, which included full blood samples from 128 people with MDD (64 of whom were also diagnosed with generalized anxiety disorder) and 64 healthy controls. Whole transcriptome sequencing was performed using the GPL10558 platform ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) [12-14].

2.2. Analyze the Data

The data analysis of this paper is carried out using R language. Batch effect removal was performed by Surrogate Variable Analysis (SVA) on the above 3 data sets [15]. The expression matrix of these data sets were downloaded. The expression matrix of ferroptosis related genes was extracted from FerrDb website (http://www.zhounan.org/ferrdb/current/). The 3 gene sets of Driver, Suppresser and Marker collected by the website were downloaded. A total of 728 genes related to ferroptosis were found [16]. Firstly, the differential expression analysis between MDD and control group was performed to find the differentially expressed ferroptosis genes. The expression matrix of differential genes was extracted. The GO terms and KEGG pathways of these genes were calculated separately. Based on the expression matrix of differentially expressed genes of ferroptosis, gene models were computed by Lasso Regression and Support Vector Machine (SVM) methods respectively. All the genes screened by the two models were intersected, and the important genes verified by the two modeling methods were obtained. Based on these genes, the diagnostic model was established by Logistic Regression method. The model was evaluated by Receiver Operating Characteristic (ROC) curve. The Gene Set Enrichment Analysis (GSEA) method was used to calculated the related pathways of each gene.

Cibersort method was used to simulate the infiltration of immune cells in MDD patients, and the reference gene set was 22 kinds of immune cell gene set [17]. The difference of immune cell infiltration between MDD and healthy control group was observed. Lnc RNA□miRNA and mRNA interactions network were mapped using cytoscape [18]. The interaction between drug and gene target was calculated by using DGIdb website (https://dgidb.genome.wustl.edu/).

2.3. Introduction of Calculation Methods and Analysis Methods Used in this Study

The R Programming Language: language and operating environment for statistical analysis and drawing. R is a free, open-source software belonging to the GNU system. It is an excellent tool for statistical calculation and statistical drawing [19]. Lasso Regression: Least Absolute Shrinkage and Selection Operator Regression. It is a method for solving linear regression models. Its main idea is that when optimizing the objective function, not only the fitting degree of regression coefficient should be considered, Also consider the absolute value of regression coefficient, so as to achieve feature selection.

Support Vector Machine (SVM): It is a kind of generalized linear classifier for binary classification of data according to supervised learning. Its decision boundary is the hyperplane with the maximum margin to solve the learning sample, which can transform the problem into a problem for solving convex quadratic programming.

Logistic Regression: It is a generalized linear regression analysis model, which is often used in data mining, disease diagnosis, economic forecasting and other fields. For example, explore the risk factors that cause diseases, and predict the probability of

disease occurrence according to risk factors. Through logistic regression analysis, the weights of independent variables can be obtained, so that we can roughly understand which factors are risk factors for diseases.

Venn Diagram: A diagram showing the overlapping areas of a collection, which is often used in mathematics, statistics, logic and other fields. Through the overlapping of shapes (usually circles or ovals), Wayne diagrams represent the intersection relation between sets, or the possibility of intersection between different sets [20]. Gene Set Enrichment Analysis (GSEA): is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes) [21].

3. Results

3.1. Batch Removal Effect

The three data sets were processed to remove batch effect, and the comparison before and after processing was shown in Supplementary. Figure S1.

3.2. Differential Expression Analysis and Enrichment Analysis

3.2.1. Differential Expression Analysis Between MDD and Control Group

The R language calculation results obtained the differentially expressed genes between MDD patients and control population, including eighteen ferroptosis genes: MAPK14, PARP9, OSBPL9, PARP4, ALOX15B, MICU1, PARK7, KLF2, QSOX1, PHF21A, MTF1, TP63, FXN, METTL14, SLC16A1, NR5A2, PRKAA1, PROK2 Figure. 1A. The relationships between the genes were calculated and demonstrated Figure. 1B.

3.2.2. GO/KEGG Enrichment Analysis

The differentially expressed ferroptosis genes were performed with GO/KEGG enrichment analysis. As shown in Figure 1C, GO terms in biological processes with relatively high enrichment degree included: intracellular receptor signaling pathway, cellular response to chemical stress, response to oxidative stress, cellular response to hydrogen peroxide, response to hydrogen peroxide, cellular response to reactive oxygen species, response to reactive oxygen species, monocarboxylic acid biosynthetic process, organic hydroxy compound biosynthetic proces, protein mono-ADP-ribosylation. KEGG pathways with relatively high enrichment degree included: FoxO signaling pathway, Fluid shear stress and atherosclerosis, Apelin signaling pathway, Alcoholic liver disease, Non-alcoholic fatty liver disease Figure 1D.

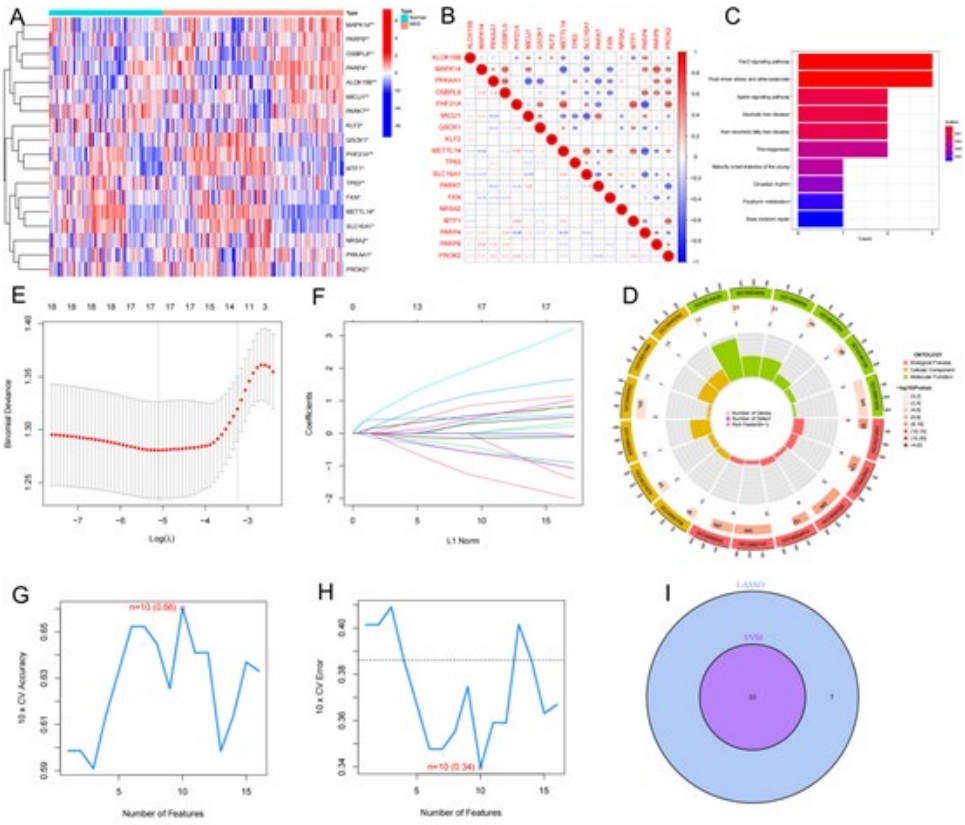

Figure 1: (A) Heat maps of differentially expressed ferroptosis genes in MDD and normal people (*p < 0.05; **p < 0.01). (B) Interrelationships among differentially expressed ferroptosis genes (*p < 0.05; **p < 0.01). (C, D) The GO terms and KEGG pathways which is enriched by the differentially expressed ferroptosis gene. Screening differentially expressed ferroptosis genes. (E, F): 17 genes are screened by Lasso regression method. (G, H): 10 genes are screened by SVM method. (I): Venn Diagram: the genes screened by the two methods are intermixed, and finally obtain 10 genes.

3.3. Establish and Validate the Ferroptosis Genes Diagnostic Model of MDD

3.3.1 The Differentially Expressed Ferroptosis Genes Were Screene

Lasso regression method and SVM method were used to further screen the expression matrix of differential ferroptosis genes, and the genes screened by the two methods were intersected Figure. 1E, F, G, H, I. The common genes were extracted, and got 10 genes: ALOX15B, MAPK14, PRKAA1, PHF21A, MICU1, KLF2, METTL14, TP63, PARK7, PARP4.

3.3.2 Model and Test the Model

Logistic regression method was used to establish the genetic diagnosis model of MDD based on these 10 genes. ROC analysis was used to validate the model and compare it with single gene diagnostic models. The results showed that the AUC was 0.773, which was much higher than the result of single gene diagnosis Figure 2A, B. The differences in the expression levels of these 10 genes in MDD and control groups were verified, and the results showed that the differences were significant and statistically significant Supplementary. Figure S2.

3.4. Gene Set Enrichment Analysis

Single-gene GSEA analysis was performed on these 10 differentially expressed ferroptosis genes to obtain the related pathways of each gene (Supplementary. Figure S3).

3.5. Analysis of Immune Cell Infiltration

The results of analysis of immune cell infiltration showed that NK cells resting and Macrophages M2 were different between MDD and control groups, and the results were statistically significant (Figure 2C). The relationship between genes and immune cells was shown (Figure 2D). In MDD patients, the gene closely related to NK cells resting was PHF21A, and the genes closely related to Macrophages M2 were METTL14 and MAPK14.

3.6. RNA Regulatory Network

LncRNA, miRNA and mRNA regulating network of 10 differentially expressed ferroptosis genes was calculated (Figure 2F).

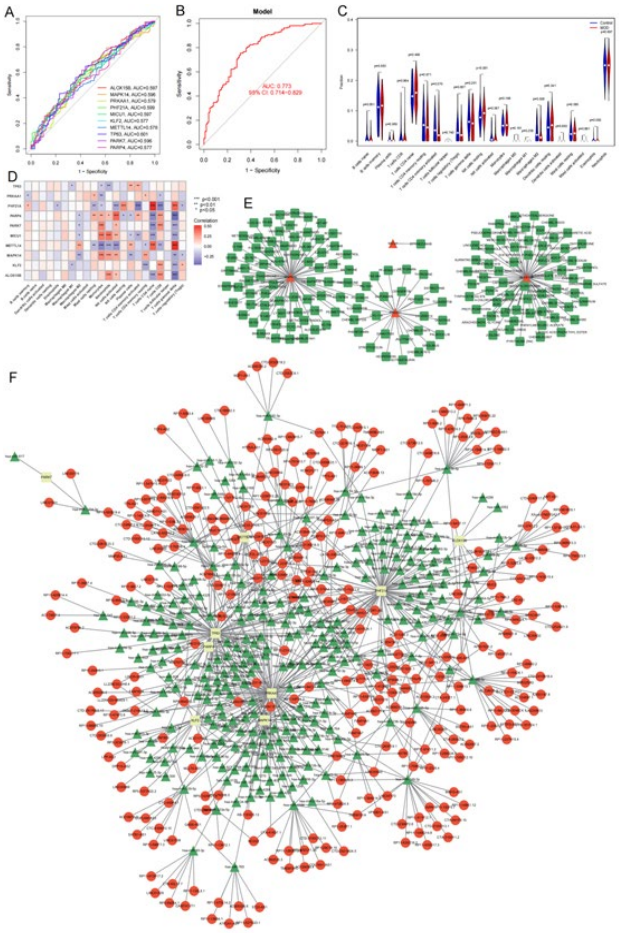

Figure 2: (A, B) ROC curve is used to validate the ferroptosis gene diagnostic model of MDD, and the result shows that the AUC of the model established by 10 differentially expressed ferroptosis genes is 0.773. The AUC of single gene model are 0.597, 0.596, 0.579, 0.599, 0.597, 0.577, 0.576, 0.601, 0.596, 0.577, respectively. (C) Correlation between immune cells and 10 differentially expressed ferroptosis genes ， *p < 0.05; **p < 0.01. (D) The analysis of immune cell infiltration. The NK cells resting and Macrophages M2 are different between MDD and control, and the results are statistically significant. (E) To predict the potential effective therapeutic drugs for 10 differentially expressed ferroptosis genes. The results showed that 4 out of the 10 genes have potential effective therapeutic drugs. (F) ceRNA regulatory network. LncRNA, miRNA and mRNA interactions regulating of 10 differentially expressed ferroptosis genes.

3.7. Screening of Potential Therapeutic Drugs

The DGIdb website was used to predict the potential effective therapeutic drugs for 10 ferroptosis genes closely related to MDD. Results 4 out of the 10 genes were identified as have potential therapeutic drugs (Figure 2E). There are 63 potential therapeutic agents for ALOX15B, 72 for MAPK14, 26 for PRKAA1, and 1 for MICU1.

4. Discussion

Major depression disorder as a mental disorder, with significant and lasting depression, loss of interest and slow thinking as the main clinical symptoms, will lead to self-injury and even suicide in serious cases. According to the 2017 statistical report of the World Health Organization, there are about 322 million depression patients worldwide, and the incidence is increasing year by year. Depression also imposes a huge financial burden on patients, families and society as a whole. According to the World Health Organization, the loss of productivity due to depression and anxiety will cost the global economy as much as $1 trillion in 2020 and the cost is still rising. Health care economists estimate that depression will become a major contributor to the global burden of disease by 2030 [22-24].

Associations between dysregulated iron metabolism and certain psychiatric conditions have been described. Most studies are based on animal models. By using quantitative proteomics, a study identifies activation of ferroptosis in mice models of depression [25, 26]. Jiao found that the content of total iron ion and ferrous ion in the hippocampus of chronic unpredictable mild stress (CUMS) model mice increased, and the regulatory factors of ferroptosis also changed significantly, which proved that there was evidence of ferroptosis in the hippocampus of depression mice. Wang found that ferroptosis in the prefrontal cortex could be reduced by reducing ferroptosis and oxidative stress in a mouse model of type 1 diabetes mellitus (T1DM), thereby alleviating its depression-like behavior [27].

In this study, the sequencing results generated from human blood samples are used as the original data to calculate the differentially expressed ferroptosis genes in MDD patients and healthy people. Further GO enrichment analysis of differentially expressed ferroptosis genes shows that the biological processes with relatively high enrichment degree include: intracellular receptor signaling pathway, cellular response to chemical stress, response to oxidative stress, cellular response to hydrogen peroxide, response to hydrogen peroxide, cellular response to reactive oxygen species, response to reactive oxygen species, monocarboxylic acid biosynthetic process, organic hydroxy compound biosynthetic proces. The results confirm that the major biological processes involved in the ferroptosis genes associated with MDD are all closely relate to the currently known ferroptosis mechanisms. Namely, reactive oxygen species catalyzed by iron cause phospholipid peroxidation of the plasma membrane and inhibit the defense system of peroxidized lipids. Lipid peroxide accumulation leads to oxidative stress response in cells, which can cause fatal damage to proteins, nucleic acids, and eventually lead to cell death [28, 29].

It is known that the main features of ferroptosis include cell morphological changes, namely the appearance of dense and shrunken mitochondria, manifested as the reduction or disappearance of mitochondrial cristogenesis and the rupture of the outer membrane. Consistent with the results of this study, the highest cell site with the GO enrichment is: mitochondrial intermembrane space.

In this study, KEGG enrichment analysis is performed on the differentially expressed ferroptosis genes between MDD patients and healthy people. The most enriched pathways include: FoxO signaling pathway, Fluid shear stress and atherosclerosis, Apelin signaling pathway, Alcoholic liver disease, Non-alcoholic fatty liver disease, confirm that most of the biological pathways of ferroptosis genes associate with MDD are related to oxidative stress and abnormal lipid metabolism.

In this study, we further analyzed the differentially expressed ferroptosis genes between MDD patients and healthy people, and screened 10 important genes. Based on these 10 genes, a MDD diagnostic model was established, and its diagnostic accuracy reached 0.773. The results show that these 10 differentially expressed ferroptosis genes (ALOX15B, MAPK14, PRKAA1, PHF21A, MICU1, KLF2, METTL14, TP63, PARK7, and PARP4) are closely related to MDD.

In this study, GSEA analysis was performed on 10 differentially expressed ferroptosis genes, and the results showed that the most frequent pathways were Parkinson's disease, Huntington's disease, and oxidative phosphorylation. These results demonstrate that ferroptosis gene plays a role in the pathogenesis of depression through three pathways: Parkinson's disease, Huntington's disease, and oxidative phosphorylation. Studies have found that ferroptosis is a key cell death pathway of dopaminergic neurons, and ferroptosis inhibitors can reduce nerve cell death in mouse models of Parkinson's disease. Even ferroptosis can be used as a therapeutic target for Parkinson's disease [30-32]. A number of studies have confirmed the pathological process of ferroptosis in animal models and human Huntington's disease patients [33-39]. The brain is more prone to oxidative stress due to its higher oxygen demand and energy demand but its weaker antioxidant capacity [40]. Oxidative stress can spread, thereby exacerbating ROS production, leading to neuronal damage. Based on the above research status, it can be speculated that the possible mechanisms of ferroptosis genes in depression through the three most enriched pathways are: nerve damage, abnormal amino acid metabolism, increased ROS, lipid peroxidation, and so on. Other pathways enriched in this study can also provide reference for follow-up related research.

Recent studies have found that ferroptosis is closely related to inflammatory immunity. For example, necrotizing inflammation has been observed in the kidneys of mouse models of ferroptosis [41]. In ferroptosis tissues, immunofluorescence staining show the presence of significantly activated macrophages [42]. There are certain inflammatory mediators produced by peroxide metabolism and arachidonic acid metabolism in ferroptosis tissues [43]. p53 can alter macrophage function by regulating

ferroptosis and plays an important role in regulating host immune responses to bacterial and malaria infections [44].

In this study, differences in NK cells resting and Macrophages M2 between MDD and healthy individuals were calculated by immune cell infiltration analysis. Further analysis of the relationship between ferroptosis genes and immune cells showed that PHF21A was closely related to NK cells resting, and METTL14 and MAPK14 were closely related to Macrophages M2 in MDD patients. Among them, METTL14 was positively correlated with Macrophages M2, and MAPK14 was negatively correlated with Macrophages M2. The negative correlation between METTL14 and MAPK14 was consistent.

Epigenetics has become one of the research hotspots in the field of psychiatry. Epigenetic mechanisms play an important role in the occurrence and development of depression [45-47]. Non-coding RNA is an important part of epigenetics. This study analyzed the RNA interaction network of ferroptosis genes closely related to depression, which provided feasible directions for subsequent related research. This study predicts potentially effective therapeutic drugs for ferroptosis genes that are closely related to MDD patients. This study provides a theoretical basis and feasible direction for follow-up related research. Of course, the conclusion of this study needs to be confirmed by further experimental research. In subsequent studies, the findings of this study can be further verified by animal experiments.

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Ethical Approval

GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Consent for Publication

Not available.

Availability of data and materials

The data that support the findings of this study are openly available in Gene Expression Omnibus database：[GEO: GSE98793] (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98793). [GEO: GSE52790] (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52790). [GEO: GSE39653](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39653).

Declaration of Competing Interests

The authors have stated that they have no conflict of interest.

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Authors' contributions

Ying Li drafted the manuscript and designed the study. Peidong Miao performed the statistical analyses. All authors read and approved the final manuscript. All authors discussed the results and commented on the manuscript.

Data statement

The data has not been previously presented orally or by poster at scientific meetings.

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