Analysis of Key Genes, Signaling Pathways, and Regulatory Networks Associated with Brain Aging based on Gene Transcriptome

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Abstract: The brain is a vital organ that governs human behavior, volition, and emotions, with brain cells serving as the fundamental structures for these activities. Research has proven that brain aging is a significant contributing factor to the decline in cognitive functions such as learning, memory, reasoning, and executive functions in older individuals. Furthermore, a series of biochemical changes resulting from cell aging are often reported as early indicators of pathological changes in neurodegenerative diseases. In an attempt to identify the key signaling pathways and core regulatory genes involved in the course of cell aging, this study deeply mined RNA-array data and RNA-seq data associated with brain aging. Firstly, differentially expressed genes highly expressed in a significant way in older individuals in comparison to younger individuals were identified, followed by enrichment analysis of signaling pathways to identify critical pathways. Subsequently, regulatory networks were analyzed on the differentially expressed genes, and finally, drug target prediction was performed for the core genes. The analysis revealed that four signaling pathways, i.e., antigen processing and presentation, inflammatory bowel disease (IBD), Bcell receptor signaling pathway and NF-kappa B signaling pathway, are closely associated with brain aging, and 20 core regulatory genes were identified, including RHOA, FYN, INSR, FOXA2, HOXA10, among others. These genes play a role in such processes as inducing cell apoptosis, regulating cell growth, and inducing inflammation. Currently, the research on brain aging and neurodegenerative diseases is not comprehensive, and there are still many puzzles yet to be solved. The findings of this study provide new research insights and directions for exploring new breakthroughs in research and understanding of brain aging.

1. Introduction

The brain is one of the most vital organs in the human body, exerting influence and control over almost all behavioral activities and volitional emotions. Brain cells, being the fundamental structures responsible for these processes, play a crucial role. Thus, the aging of brain cells leaves a profound impact on life activities of human beings. Research indicates that brain aging is one of the significant factors contributing to the decline in cognitive functions such as learning, memory, reasoning, and executive functions in older individuals[1]. A suite of biochemical changes incurred by cell aging are often reported as early indicators of pathological changes in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Down syndrome (DS), and multiple sclerosis (MS)[2]. Due to the strong correlation between many diseases and age, as research advances, there is an increasing emphasis on exploring cell aging and gaining a deeper understanding of the impact of brain aging on life activities and health of human beings.

The gradual decline in cellular proliferation, differentiation capability, and physiological functions over time, during the execution of life processes, is referred to as cell aging. For a long period of time, the triggering factors of cell aging remained unclear. However, thanks to the continuous advancement of research techniques of life science, we have gained a deeper understanding of the mechanisms underlying cellular senescence and the resulting changes in tissue health. On a macroscopic level, the gradual reduction in brain volume, cortical thinning, and enlargement of ventricles, etc. have been identified as hallmark features of brain aging [3]. On a microscopic level, it has been discovered that a repetitive nucleotide sequence of TTAGGG, known as telomeres, located at the ends of chromosomes, plays a crucial role in replicative senescence as its attrition is considered a molecular determinant[4]. Furthermore, research has demonstrated that even if aging is triggered by different initiating events, the aging growth arrest associated with senescence requires the coordinated action of tumor suppressor pathways of the p53/p21 and p16INK4A/retinoblastoma (RB)[5]. In recent studies, a signaling pathway activated in response to endoplasmic reticulum stress, known as the unfolded protein response (UPR), has been found to play a significant role in brain degeneration of mammals. In mouse models, the active

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form of the transcription factor XBP1, which exhibits high expression of UPR, has been shown to restore synapses and cognitive function while reducing cell aging simultaneously. These findings suggest that manipulating the UPR pathway in mammals may exert a significant impact on maintaining brain health during aging[6].

The emergence of gene chip and other technologies, coupled with the development of second-generation gene sequencing technology, has allowed us to gain a more nuanced understanding of the signaling pathways implicated in cell aging. Therefore, the aim of this paper is to mine the gene transcriptome data relevant to brain aging, perform an analysis of the involved signal transduction pathways, discover the key pathways that govern cell aging, assess their concreteity in relation to brain aging, identify concrete indicators for diagnosing brain aging, and investigate the potential of these pathways as targets for drug therapy, thus providing fresh insights for drug development.

2. Dataset and Method

2.1. Datasets

The relevant literature and gene data mentioned in this paper were retrieved from PubMed and GEO DataSets. The study results and high-quality gene transcriptome data selected for this paper were derived from research conducted on human beings and macaques. Specifically, the data were collected from a study published by Kenneth L Chiou et al. in 2022 (GSE179328)[7] and a study published by Tao Lu et al. in 2004 (GSE1572)[8].

2.2. Analysis of Differentially Expressed Genes

To investigate the key genes involved in the process of brain aging and the differential expression of these genes between young and elderly individuals, the GDS707 (GSE1572) dataset in the GDS database was partitioned into two groups: the elderly group and the young group. Moreover, the GSE1572 dataset was subjected to analysis of two groups using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE1572), and the data for gene symbol, gene ID, adjusted P-value, P-value and T-statistic of each gene as well as log2 (FC) (difference multiple) was extracted. Genes with P-value < 0.05 and absolute value of log2 FC (old v.s. young) greater than 1 are considered as differentially expressed genes.Similarly, gene data from the M1 region of GSE179328 were selected, the data for mean, FC, log2(FC), and Pvalue of gene expression were calculated, and genes with a Pvalue < 0.05 and absolute value of log2FC (old v.s. young) greater than 0.5 were selected as differential genes.

2.3. Enrichment of Signal Pathways

The differentially expressed genes calculated from the GDS707(GSE1572) dataset were enriched and analyzed in Kobas (http://kobas.cbi.pku.edu.cn/genelist/) and reactome (https://reactome.org/). The pathway with the corrected P-value < 0.05 was selected from the enrichment results of kobas. In reactome (https://reactome.org/), pathways were also subjected to enrichment analysis, concreteally targeting those with a P-value < 0.05. The intersection of enrichment results from two databases was computed. Likewise, differentially expressed genes from GSE179328 were selected and analyzed for enrichment in kobas. A comparison was made with the enrichment results from GSE1572, and the intersection was determined. The concrete signal transduction pathways associated with the differentially expressed gene expression was visualized and the signaling processes and distribution locations of the differentially expressed genes within these pathways was determined by using KEGG mapper.

2.4. Analysis of Transcriptional Regulatory Networks

The differentially expressed genes of human beings and macaques were analyzed in networkanalyst (https://www.networkanalyst.ca/) and the regulatory network was analyzed based on the SIGNOR 2.0 (https://signor.uniroma2.it/) database. The top ten most key genes were selected for visualization.

Prediction of Potential Drug Targets

Similarly, the differentially expressed genes between macaques and human beings were analyzed in networkanalyst, and the possible drug targets were identified by means of protein-drug interaction. The drug database came from drug bank (https://www.drugbank.ca/), and the top ten targets with the highest correlation were selected for visualization.

3. Results

3.1. There are significant increases in the transcription levels of a large number of genes in aging brain tissue

To systematically screen the genes associated with brain aging as well as their signal pathways, we first chose the RNA-seq data (GSE179328) in the article[7] published by Kenneth L Chiou et al. in Nat Neurosci in 2022 and the RNA-array data (GSE1572) involved in the article[8] published by Tao Lu et al. in Nature in 2004. In GSE1572, we chose the data of M1 area, which is the first body movement area of the brain, and principally regulates the movement of skeletal muscles. Besides, the MI cortex is not merely a static motion control structure, but contains a dynamic matrix, which engages in motor learning or cognitive events[9]. Parkinson's disease (PD) is a common degenerative disease of the brain, and its motion abnormality is triggered by the disorder of neural activity in numerous interrelated brain structures. The planning and execution of motion need to recruit a heterogeneous collection of pyramidal projection neurons in the primary

motor cortex (M1). In the recording of single cell and field potential of M1, the neural representation of movement is directly or indirectly influenced by dopaminergic neurons of the midbrain, which will degenerate in PD[10]. The article suggests that M1 plays a vital role in degenerative diseases of the brain.

Firstly, in GSE179328 (gene data of macaques), there were totally 36 macaque samples, of which 20 were females and 16 were males. They were grouped according to age, 0~10 years old for the young group, 10~20 years old for the old group, each with 18 samples. The mean, FC, log2FC (old group v.s. young group) and P-value of each gene expression in the old group and the young group were calculated respectively. It was considered that the genes with log2(FC) greater than 0.5 and p-value less than 0.05 were apparently different genes, and they were highly expressed in the old group and lowly expressed in the young group. A total of 97 differentially expressed genes with significantly high expression in the old group were screened out (Figure 1A).

In Gene1572 (gene data of human beings), there were 30 human samples in total, of which 12 were female and 18 were male, which were analyzed in GDS database. The screening conditions were set as T-test A > B, and the P-value was less than 0.05. The samples were divided into old group and young group. Samples aged 20-39 were classified into the young group, and samples aged 80 and above were classified into the old group for analysis. There were 1062 genes with obvious expression differences (Fig 1B) after choosing the data source as Gene.

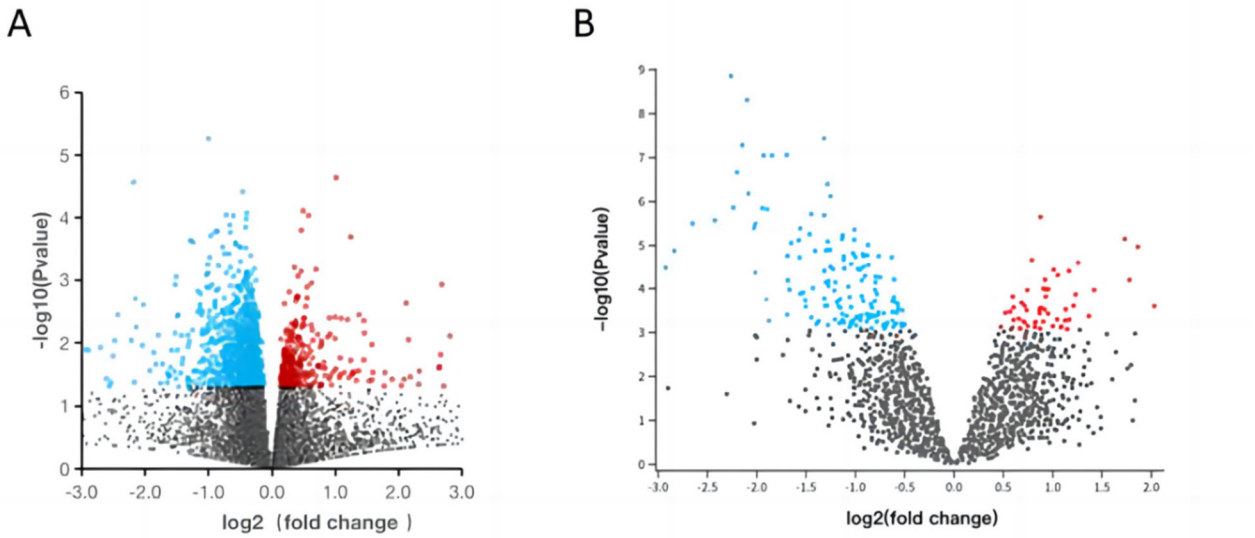

Figure 1. Analysis of differential gene expression in the brain of old and young age groups. Volcano plot of expression profiles from GSE179328(A) and GSE1572(B), as determined by RNA-seq and RNA-array analysis. The horizontal coordinate is log2 (FC) and the vertical coordinate is -log10 (Pvalue). Red dots are data for RNAs that were significantly more highly expressed in the old group than in the young group (Pvalue<0.05, log2FC>0.5). Blue dots are data for RNAs that were significantly lower expressed in the old group than in the young group(Pvalue<0.05, log2FC<-0.5). Grey ones are non-significant data (Pvalue>0.05).

3.2. Brain aging relates to abnormal activation of multiple signal pathways

Based on the differentially expressed genes screened by the above analysis, we further analyzed the signal pathways associated with brain cell aging. The results of enrichment analysis of differentially expressed genes by KOBAS revealed that there were 46 effective pathways with P-value less than 0.05 in KEGG PATHWAY and Gene Ontology for gene data of macaques, and 2,484 effective pathways with P-value less than 0.05 in KEGG PATHWAY, Reactome and Gene Ontology for gene data of human beings. The top ten pathways enriched by macaques (Fig 2A) with the strongest correlation were chosen to look for co-existing pathways in human pathways (Fig 2B). It was learned that the four signaling pathways, antigen processing and presentation, inflammatory bowel disease (IBD), Bcell receptor signaling pathway and NF-kappa B signaling pathway, were all significantly up-regulated in the brain of the old group.

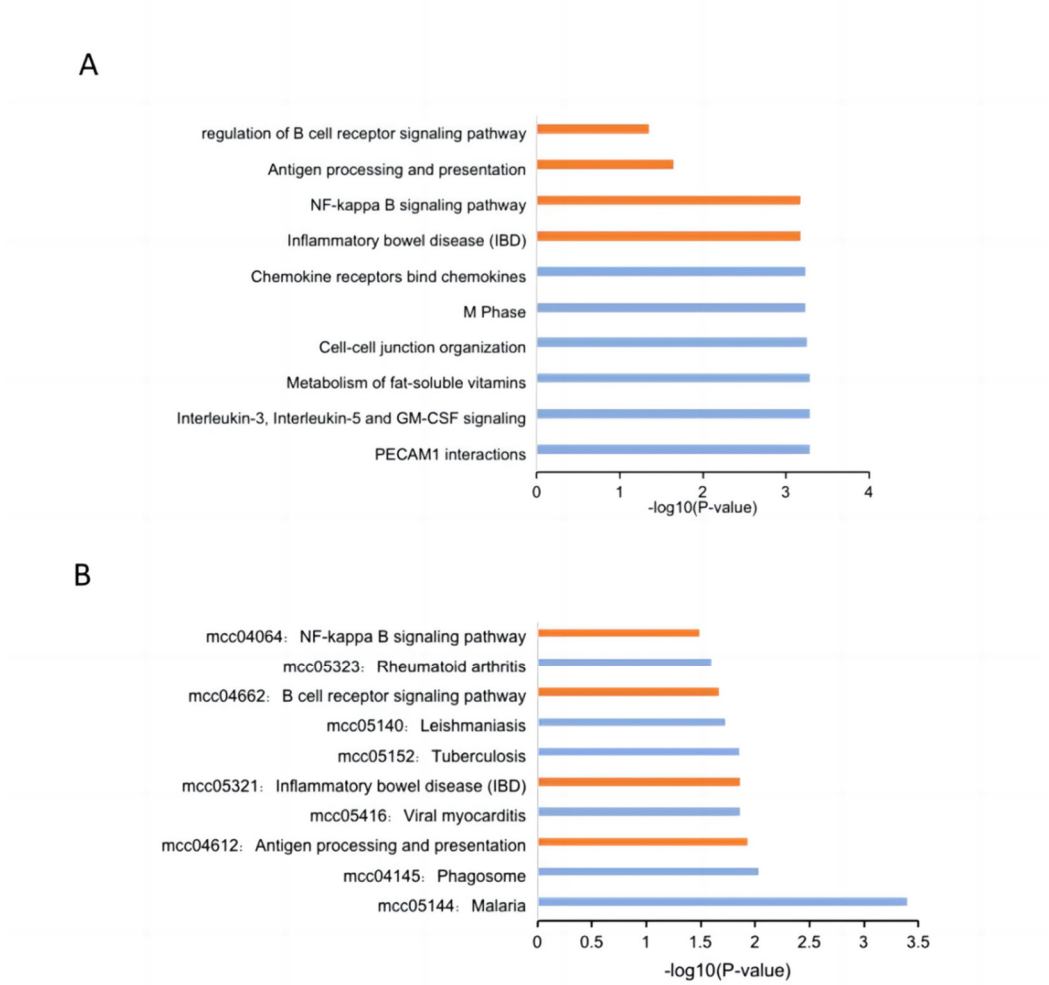

Figure 2. Signalling pathway analysis of differential genes. The bar chart showed the top ten signalling pathways obtained from the KEGG enrichment of differential genes in GSE179328(A) and GSE1572(B) datasets, the orange bars are the overlapped signalling pathway in two datasets.

3.3. Analysis of Regulatory Networks of Key Genes in Brain Aging

Furthermore, we analyzed the regulatory networks of genes with significantly high expression in the aging brain, introduced the differentially expressed genes of human beings and macaques into networkanalyst, and performed an analysis based on SIGNOR2.0 database. It was concluded that there were 32 subnetworks involved in human beings, among which the top ten genes were RHOA, ESR1, PLK1, HDAC1, FYN, BCL2, INSR, PDPK1, HIF1A and BRCA1 (Fig 3A). Therein, research has reported that RHOA encodes a member of the Rho family of small GTPases, which cycle between inactive GDP-bound and active GTP-bound states and function as molecular switches in signal transduction cascades. The activity dysregulation of GTP enzyme in Rho family is a common feature in the process of degeneration. It is involved in cytoskeleton regulation and cell death. Moreover, recent articles indicate that it is linked with neurodegeneration-related cell processes, e.g. mitochondrial homeostasis, autophagy and neuroinflammation[11]. Apart from that, there was increasing evidence that the kinase FYN in Src family was associated with the pathological physiology of AD[12]. INSR (i.e., IR) is an insulin receptor, and the defects of IR activation, insulin availability and downstream IR-related mechanisms may result in IR-mediated dysfunction, which will subsequently cause a wide range of brain diseases, covering neurodevelopmental syndrome, tumors, neurodegenerative diseases and depression[13].

There were 15 subnetworks in the regulatory network of macaques, among which the top ten were HOXA10, TLR4, FOXA2, TBX21, PBK, RAC2, TNFRSF13C, MUSK, SIX1 and ATF3(Fig 3B), where HOXA10, FOXA2, TBX21 were the common genes of human beings and macaques. Nurr1 and FOXA2 were transcription/epigenetic regulators, which can cooperate to regulate the inflammation and neurotrophic response of glial cells. The joint expression of Nurr1 and FOXA2 tremendously improves the AD associated with amyloid β and Tau proteinosis, cell aging, synaptic loss and neuroinflammation in all types of AD models in vitro and in vivo. The adeno-associated virus (AAV) serotype 9 was used to deliver the Nurr1 and Foxa2 genes into the brain, which improved the memory and cognitive function of mice by AD model[14].

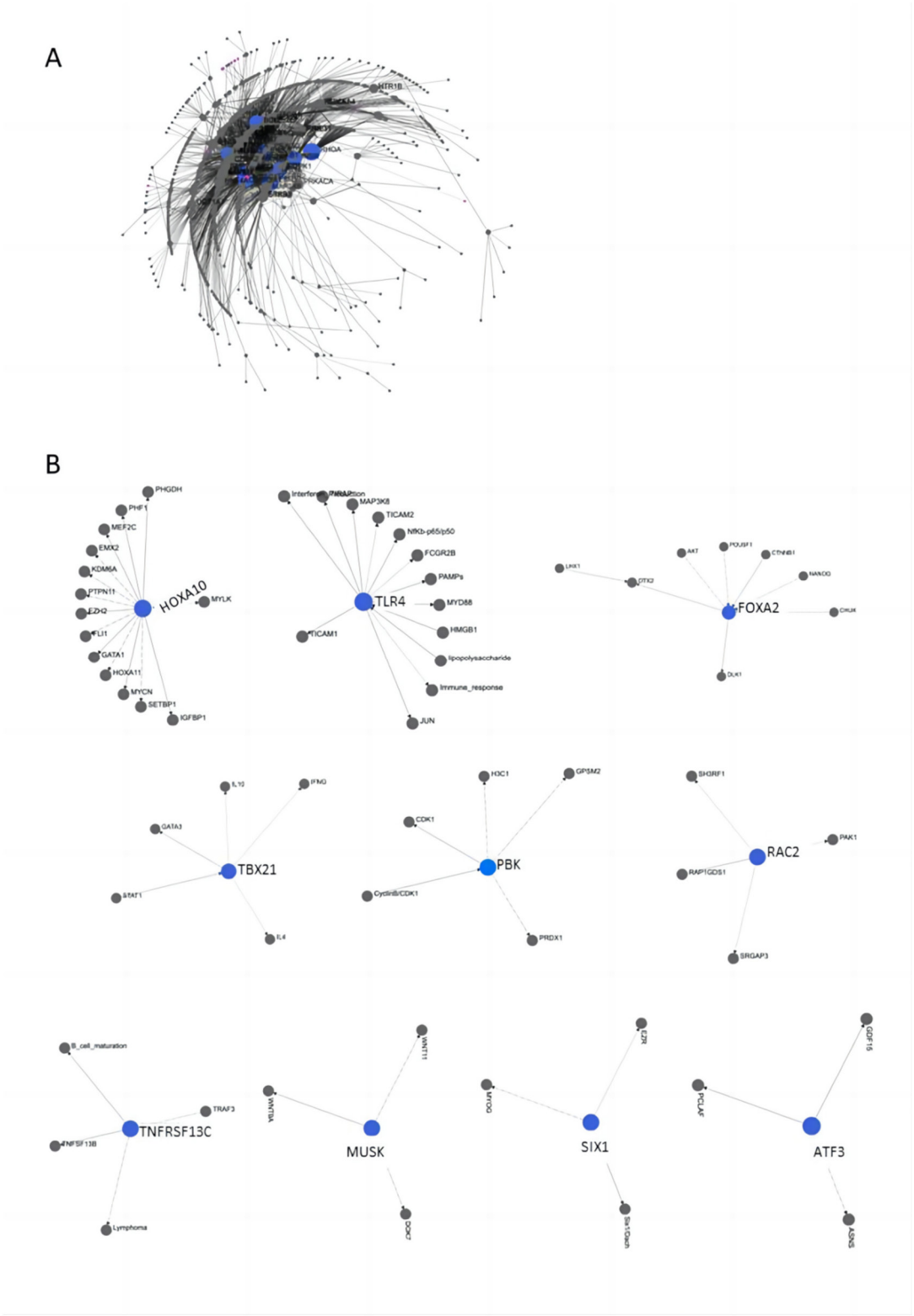

Figure 3. Gene expression regulatory network analysis. Regulation network map from networkanalyst, each dot represents a gene. Map obtained from differential genes of GSE1572(A), and the blue ones are the most significant genes in this regulation map. Maps obtained from GSE179328(B), we have selected 10 core genes from 15 subnetworks, they are marked as blue dot.

3.4. Prediction of Possible Drug Targets

46 core genes were screened out based on the above four signal pathways as well as the regulatory networks of human beings and macaques; the drug targets were further analyzed in networkanalyst; protein-drug interaction was chose for analysis based on drug bank; a total of 11 subnets were acquired; the top ten drug targets were also screened out, namely, IL-2, IFNG, PDPK1, PRKCQ, INSR, BCL2-2, TLR4, HDAC1, PLK1 and HIF1A (Figure 4). These targets may exert an impact on the process of human brain aging by manipulating key signal pathways or regulatory networks associated with human brain aging under the action of drugs.

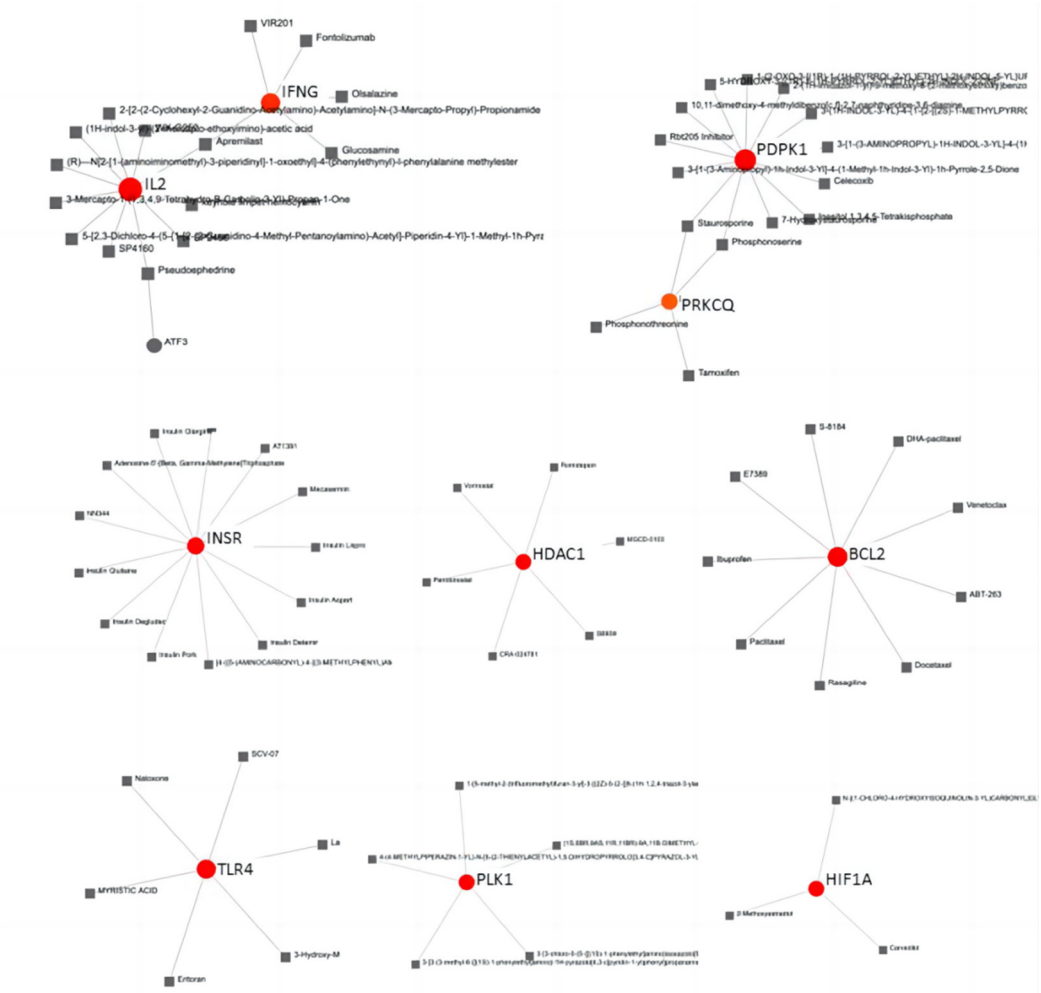

Figure 4. Prediction of drug interaction. The figure above shows the drug targets obtained from the analysis of key genes in the signalling pathways and regulatory networks,based on networkanalyst.Square icons indicate drugs,the circle icon indicates a gene,we marked the core genes in red.The ten core genes were selected from 11 subnetworks.

4. Discussion

The human brain, being the central hub of human life activities, is both crucial and intricate. Research has reported that cell aging is an inevitable process, but the brain possesses additional anti-aging systems. Without these systems, the rate of normal aging could potentially be significantly higher[15]. The research on this anti-aging system is still not fully understood. This study is intended to identify key signaling pathways and regulatory genes involved in the aging process of the human brain, with a view to regulate the aging and anti-aging processes by exploring and intervening in these key signaling pathways and regulatory genes, with the objective of slowing down aging and treating pathological aging.

In this study, the RNA-seq data of macaques (GSE179328) and the RNA-array data of human beings (GSE1572) were analyzed, revealing significant differences in gene expression between the high and low age groups within the datasets. Specifically, the expression levels of certain genes were significantly higher in the high age group in comparison to the low age group. Based on these differentially expressed genes, four signaling pathways were enriched that were shared between macaques and human beings, respectively antigen processing and presentation, inflammatory bowel disease (IBD), B cell receptor signaling pathway and NF-kappa B signaling pathway. Furthermore, an analysis of the regulatory networks of key genes was conducted, identifying core genes such as RHOA, ESR1, and PLK1. Possible drug targets were predicted, including IL2, IFNG, and PDPK1, along with the prediction of compounds and interaction networks associated with their interactions.

Within the NF-kappa B signaling pathway, the high expression of cIAP1/2 activated RIP1, which in turn activated IkBa, leading to the transcription of NFkB-dependent genes, including pro-inflammatory factors, growth factors, and chemokines, etc.. The study conducted by Tan D et al. revealed a declining trend of 3-sulfo-galactosylceramide (SGDG) in the central nervous system with increasing age. When SGDG acted on the NF-kappa B signaling pathway, it was found to inhibit gene expression induced by LPS, as well as the release of pro-inflammatory cytokines from macrophages and microglia. This confirmed the evolutionary protective role of SGDG in the brain[16]. Thus, with the decline in SGDG levels as age increases, the weakening of the brain's protective mechanisms makes it more susceptible to aging and pathological changes. This study further corroborates the importance of the NF-kappa B signaling pathway, as predicted by us, in the regulation of brain aging.

The research on the mechanisms and treatment of IBD has been quite extensive, and multiple studies have demonstrated the association between IBD and depression and anxiety, as well as the sequence of occurrence of these two types of disorders. Furthermore, possible mechanisms underlying the co-occurrence of IBD with depression and anxiety have been identified, including changes in brain signaling and morphology, increased levels of pro-inflammatory cytokines in the peripheral and central nervous systems, and alterations in vagal nerve signaling[17]. The brain-gut axis has been a hot topic of research in recent years. It was previously believed that the gastrointestinal tract was an organ controlled by the central nervous system. However, recent studies have indicated that the gastrointestinal tract has its own independent neural structures, which play a dual role in the regulation of intestinal function, both in terms of incoming and outgoing signals. This demonstrates the bidirectional interaction between the brain and the gut, giving rise to the concept of the brain-gut axis. The aforementioned research on the association between IBD and depression and anxiety further substantiates the existence of this connection. New data suggests that gut microbiota may shape neurodevelopment, regulate neurotransmitters, and influence behavior, thereby contributing to the onset and/or progression of numerous neurodevelopmental, neuropsychiatric, and neurological disorders[18]. The article also mentions that introducing therapies that impact the gut-brain axis may alleviate symptoms of Parkinson's disease. This suggests that the brain-gut axis may play a role in brain aging and that targeting the brain-gut axis could be a therapeutic approach for brain disorders. The research on antigen processing and presentation primarily focuses on immune-related diseases, as well as the mechanisms and treatment of tumors. Studies on the Bcell receptor signaling pathway also predominantly concentrate on its role in the mechanisms and treatment of cancer. However, there is limited detailed research on the involvement of these pathways in brain aging.

In the search for drug targets, a compound called glucosamine, which acts on IFNG, has been discovered. There is evidence suggesting that glucosamine reduces inflammation by inhibiting interferon gamma γ15, 16, 17, and NF-κBp65. Clinical trials primarily focus on its association with osteoarthritis and pain management, but further investigation is needed to explore the effects of this compound on chronic brain inflammation. However, experimental studies on compounds targeting these core genes are still pending.

In conclusion, a systematic mining of transcriptomic data on brain aging was conducted in this study, progressing from differentially expressed genes and signaling pathways to the identification of core genes in regulatory networks. The focus was narrowed down to these core genes, and predictions were made regarding their potential as drug targets. Furthermore, a summary of the current research status on key signaling pathways and the physiological processes involving these core genes was provided. Future experimental validations will provide more reliable support for the findings of this study.

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