Analysis of related pathways of gene expression difference in muscle tissue of Alzheimer's disease mice

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Abstract: Objective: To analyze the differentially expressed genes in muscle tissue of Alzheimer's disease (AD) model mice and reveal the pathogenesis of AD from transcriptome level. Methods: The control group and AD group were set up, and the differential expression of gene transcriptomics in the model group was detected. Through functional annotation and enrichment analysis of mouse gene sequencing in the model group, genes related to the pathological mechanism of AD due to kidney deficiency and metabolic pathways were screened out. The STRING database and Cytoscape software were used for protein-protein interaction network analysis. Results: GO analysis showed that DEGs were mainly distributed in the cytoplasm, membrane and extracellular space, and could induce AD through positive/negative regulation of transcription, positive regulation of nuclear factor κB activity, and other molecular functions. The up-regulated genes of muscle tissue expression in SAMP8 mice are mainly located in the ribosome and mitochondria of cells. The main biological processes are protein synthesis, sorting and localization in cells as well as viral nucleic acid synthesis. The main molecular function is NADH reductase activity. The results of KEGG pathway enrichment analysis indicated that these genes participated in important biological pathways such as phagocytosis, lysosomes, Toll-like receptor signaling pathway, cytokine receptor interaction, and NF-κB signaling pathway. Conclusion: The AD-related differentially expressed genes provide experimental basis for AD-related mechanisms and treatment research in model mice.

1. Introduction

Alzheimer's disease (AD) is a degenerative disease of the central nervous system, with progressive memory cognitive impairment and intellectual disability as the main clinical manifestations, and it is the most common dementia disease. As people live longer, the number of people suffering from the disease will become larger and larger. Pathological changes in AD are associated with senile plaques and neurofibrillary tangles formation in the brain. The possible mechanisms include deposition of β -amyloid peptide, inflammatory injury, and oxidative stress. The main involved sites were cortex and hippocampus. The neuroimaging observation of 118 AD patients revealed that the mental symptoms such as delusion, indifference, and depression commonly seen in AD patients were closely related to the muscle tissue [1]. At present, the disease incidence in China has reached 10 million people. With the continuous increase of the aging population, the incidence of AD continues to increase [2–3]. AD can be classified into the category of "dementia" according to the clinical manifestations of AD patients [4].

Therefore, revealing the unique aging pattern of AD is conducive to clarifying the mechanism of aging in the occurrence and development of AD. Big data-based systems biology analysis offers the possibility to qualitatively analyze aging patterns of AD. SAMP8 mouse is a typical rapid aging dementia mouse, and it can appear the typical rapid aging characteristics accompanied by dementia in adulthood, which is the commonly used AD animal model [4]. From January 2019 to December 2020, we analyzed the differentially expressed genes in muscle tissue of SAMP8 mice and SAMR1 mice, and modeled the LINCS database to analyze the characteristics of differentially expressed genes in muscle tissue of rapid aging dementia mice and predict the therapeutic drugs for senile

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dementia. This is reported below.

2. Materials and methods

2.1. Animal

Twenty three-month-old healthy male SAMP8 mice were provided by Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (CertificateNo.: SCXK (Beijing) 2016-0002). Animal experiments were performed in the Laboratory Animal Department of China Medical University [animal use permit No: SYXK (Liao) 2015-0001].

2.2. Main reagents and instruments

Trizol is purchased from Invitrogen, the US; DEPC water was purchased from QIAGEN, the US; Prime script tm rt mastermix kit was purchased from Takara, japan; The Ethovision XT animal tracking and behavioral observation record analysis system was purchased from Noldus, the Netherlands. Ultra SYBR mixture (with ROX) was purchased from Takara, Japan. Hi Seq 3000 sequencer was purchased from Illumina, the US; Primers were purchased from Invitrogen, China; Qubit 2. 0 quantitative instrument was purchased from Invitrogen Co., Ltd., the US. Agilent 2100 is purchased from Agilent, the USA; PCR instrument was purchased from Bio-Rad, the US; ABIstepone fluorescent quantitative PCR instrument was purchased from Thermo, the USA.

2.3. Method

2.3.1. Animal grouping

Twenty three-month-old healthy male SAMP8 mice were adaptively fed for one week and randomly divided into two groups: control group and AD group.

2.3.2. Preparation of animal model

The STZ-injected mice were anesthetized with tribromoethanol and fixed on the brain stereotaxic apparatus. The skin was routinely sterilized through a sagittal incision, and the periosteum was separated. The skull was drilled through a cranial awl to expose the dura mater. Six weeks after injection, the mice were sacrificed by decapitation. The cerebral cortex, hippocampus, cerebellum, and brain stem were rapidly separated and stored fresh on dry ice at-80 C for later biochemical studies. Some muscle tissues were fixed in 0.1MPBS containing 0.4% paraformaldehyde, and 30% sucrose ultra-low temperature protective agent was added. Sagittal sections were obtained using a slide microtome. Sections will be stored in ethylene glycol antifreeze at-20 C for immunohistochemical staining.

2.3.3. Extraction of differentially expressed genes in muscle tissue of SAMP8 mice

The 2-month-old and 8-month-old SAMP8 mice were sacrificed by cervical dislocation. The muscle tissues were taken and put into a pre-cooled glass homogenizer for homogenization. The total RNA in cells was extracted by TRIzol method, and identified by the ultraviolet spectrophotometer. The concentration and purity of the extracted total RNA were qualified. Three RNA samples were reserved for each month-old mouse to make gene chips. Probe synthesis, chip hybridization and washing and dyeing, chip scanning, and preliminary data analysis were all completed by Beijing Braunscom Technology Co., Ltd.

2.3.4. Extraction of total rna in muscle tissue

The total RNA in the prefrontal cortex was extracted by Trizol method, and the purity, concentration and integrity coefficient of RNA samples were detected by Nanodrop, Qubit 2. 0 and Agilent 2200, respectively, to ensure the sequencing using qualified samples.

2.3.5. Genomic alignment and differentially express gene analysis

The sequencing data were compared with the mouse reference genome GRCm38 by the software

Tophat2, and a bam file was obtained, in which the chromosomal location of each reads was recorded. The genes were quantified using DESeq software. EdgeR software was used to analyze the significance of expression differences and clustering analysis was performed on the expression values of AD samples.

2.3.6. Statistical analysis

SPSS19. 0 software was used for analysis and the homogeneity test of variance and one-way analysis of variance were used for comparison between two groups. The difference was statistically significant if $P < 0.05$.

3. Result

3.1. Transcriptome data evaluation

The transcriptome analysis of two samples was completed. The analysis of filtration quality control results of transcriptome data showed that the overall quality of the data obtained after sequencing filtration was high, the percentage (Q30) of bases with mass value ≥30 was 95. 44% and above, the proportion of GC sequences was 51.24%, A and T were approximately equal, and C and G were approximately equal.

3.2. Results of metabolic pathway analysis of rapid aging-related gene KEGG

In the gene expression pattern of muscle tissue in SAMP8 mice, the KEGG metabolic network pathways with statistically significant 747 up-regulated genes were mainly mitochondrial oxidative phosphorylation pathway, three neurodegenerative disease metabolic pathways (AD, Parkinson's disease and Huntington's disease), ribosomal protein synthesis pathway, non-alcoholic fatty liver disease, and E. coli infection metabolic network pathway. There was no significant difference in the analysis results of 235 down-regulated genes. In the gene expression pattern of muscle tissue in SAMR1 mice, there was no significant difference in KEGG metabolic network pathway of 115 up-regulated genes and 533 down-regulated genes (Table 1).

Table 1 Results of KEGG metabolic network pathway analysis of up-regulated gene expression in muscle tissue of SAMP8 mice

Serial number	KEGG metabolic network pathway	P	Correction p	Z
1	Oxidative phosphorylation	8.21E-12	1.97E-10	-1.75
2	AD	8.44E-10	9.83E-11	-1.87
3	Parkinson's disease	1.30E-11	1.17E-9	-1.72
4	Huntington's disease	5.58E-8	1.31E-7	-1.83
5	Ribosome	2.14E-9	1.33E-7	-1.55
6	Non-alcoholic fatty liver	1.43E-6	5.58E-4	-1.75
7	Escherichia coli infection	2.28E-5	7.76E-4	-1.88

3.3. GO analysis and KEGG pathway enrichment analysis results of DEGs

The results of GO analysis showed that DEGs was mainly involved in positive/negative regulation of transcription, positive regulation of nuclear factor κB(NF-κB) activity, regulation of Rho protein signal transduction, jaw development, regulation of protein phosphorylation, negative regulation of autophagy, and other biological functions. DEGs were mainly distributed in cytoplasm, membrane, extracellular space, Golgi apparatus and other cellular components. DEGs mainly involves molecular functions such as protein binding, DNA binding, transcription factor activity (sequence-specific DNA binding), and protein homodimer activity.

The KEGG pathway enrichment analysis results showed that DEGs was significantly enriched in signaling pathways such as cancer pathway, tuberculosis, osteoclast differentiation, Janus kinase/signaling and transcriptional activator (JAK/STAT) signaling pathway, forked transcription factor (FoxO) signaling pathway, Epstein-Barr virus infection, and transforming growth factor

β(TGF-β), as shown in Fig. 1.

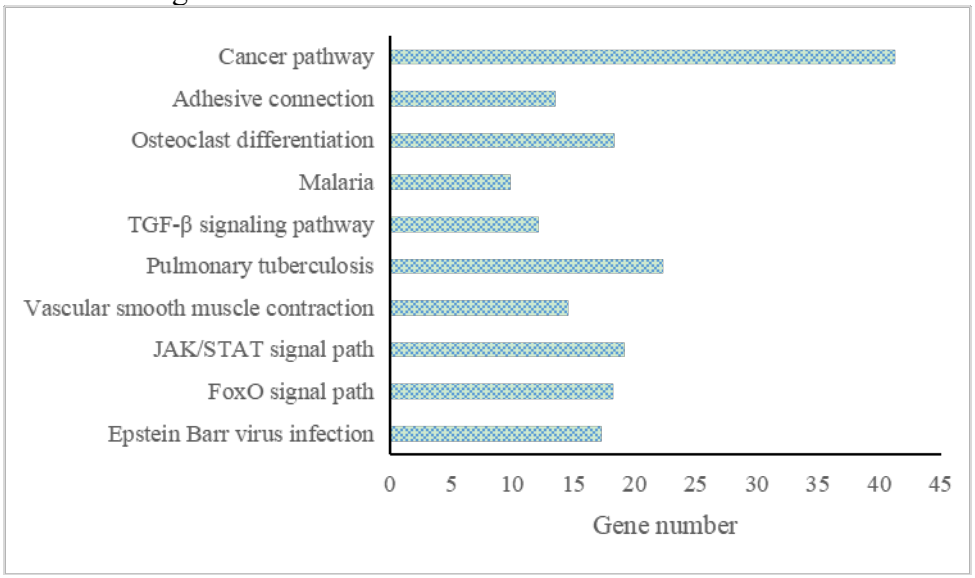

Figure 1 Results of KEGG pathway enrichment analysis of DEGs

3.4. Comparative analysis of gene expression changes between hippocampus and cerebral cortex

We quantified the expression of AD-related genes in the cerebral cortex of laboratory rats and compared the differences in gene expression changes in the hippocampus and cerebral cortex. We found that in the two brain regions, although the gene changes in some regions are not statistically significant, the trends of these gene changes are the same. In general, there were statistically significant changes in more genes in the cortex relative to the hippocampus in STZ-injected rats, whereas there were more positive changes in the hippocampus than in the cortex in the tri-transgenic rats. These results suggest that changes in different brain regions were also different for the expression of AD-related genes in STZ-injected and tri-transgenic mice.

4. Discussion

AD is a progressive nervous system degenerative disease with slow onset and relatively hidden manifestations, mainly including changes in memory, language, behavior, and personality. Until now, the etiology is still unclear. In contrast, AD is mainly recognized in traditional Chinese medicine due to senile body deficiency, deficiency of essence and qi, as well as ocean of marrow failure, thus leading to the failure of vital energy utilization. Memory impairment is the first symptom of AD. Kidney deficiency is closely related to AD. Kidney essence is deficient, and deficiency is the foundation. Therefore, treatment of AD in TCM should consider treatment based on kidney.

With the rapid development of transcriptomics sequencing technology, transcriptomics is deeply loved by scientific research workers because of its characteristics of systematicness, integrity and high efficiency at the whole gene transcription level. Through mining, analyzing and summarizing the pathological basis related to the disease and elaborating the possible action mechanism of the active components of drugs on the basis of molecular level, transcriptomics is able to reveal different syndrome manifestations due to the different biological effects of gene expression into protein. The biological basis of syndrome manifestations is found through transcriptomics sequencing technology [5]. In this study, the AD mice with kidney deficiency were analyzed for the possible potential pathogenic mechanism and related pathogenic genes by transcriptomics. The results of difference analysis in this experiment as well as GO and KEGG analysis showed that the genes involved in cellular processes and the components of cells were significantly different.

The results of GO analysis and KEGG metabolic pathway analysis of the up-regulated gene expression in muscle tissue of SAMP8 mice both showed significant concentration and enrichment

characteristics. The cellular localization of the up-regulated gene expression was mainly ribosomal and mitochondrial, suggesting that a large number of mitochondrial-related protein were synthesized through ribosomal translation. Its main biological functions are the synthesis, sorting and localization of protein in cells. The results of GO bioinformatics analysis suggested that the dementia-related gene expression pattern in SAMP8 mice was a special cell mitochondrial repair pattern. Results from KEGG metabolic network pathway analysis also support this viewpoint. The metabolic pathways with statistical significance also included the ribosomal protein synthesis pathway and three neurodegenerative disease metabolic pathways, indicating that the gene expression pattern related to dementia in SAMP8 mice had the typical characteristics of neurodegenerative disease pattern.

Developmental inhibitory protein 4(NEDD4), an important HECT-type ubiquitin ligase, is encoded by NEDD4 gene and highly expressed in tissues related to a variety of neurodegenerative diseases [6]. NEDD4 gene has important biological functions in the process of body development and disease progression, and it can bind to a variety of substrates and regulate a variety of signaling pathways. It has been found that the NEDD4 enzyme can regulate nerve and embryonic development by ubiquitination of fibroblast growth factor receptor 1(FGFR1) [7]. Studies [8] have shown that NEDD4 enzyme is involved in the occurrence and development of a variety of neurodegenerative diseases through ubiquitination regulation and acquired immune regulation, serving as one of the potential targets for studying the pathogenesis of AD. The results of this study have shown that the NEDD4 gene is mainly concentrated in the anti-reactive energy group (GO0031623) against calcium. However, further studies are needed to investigate how this gene regulates the calcium responsive functional group and its role in the development of AD.

In the present study, we found that the expression levels of Slc2a3 and Slc2a4 in the cortex of the STZ injection rat, the cortex of the tri-transgenic rat, and the hippocampus were significantly decreased. These two genes code for GLUT3 and GLUT4, respectively, so these two genes are important for the glucose absorption by neurons. Recently, more attention has been paid to the effects of GLUTs on the pathogenesis of AD [9]. The main functional characteristics of GLUT are its saturation, structure specificity, non-energy consumption and competitive inhibition. In mammals, glucose transport and uptake are assisted by GLUT. The content of GLUT3 in the brain of AD patients is greatly reduced, causing disorders of energy metabolism in brain neuronal cells and leading to neuronal degradation, thus leading to the corresponding clinical symptoms in AD patients. Besides, studies have confirmed that this phenomenon does not arise from neuronal degeneration, which leads to the decrease of GLUT3 content, but rather from the decrease of the latter, which leads to the degeneration of the former. This conclusion further proves that the reduction of glucose metabolism plays an important role in the occurrence and development of AD.

5. Conclusions

In summary, in this study, the highly correlated differential genes in muscle tissues of AD model mice were obtained by RNA-Seq, which provided an important experimental basis for elucidating the pathogenesis of AD.

Acknowledgements

Research in the molecular mechanism that involved in PI3K/AKT, ERK1/2 and P75NTR in inter action the therapy of rat Alzheimer’s disease by breviscarpine injection

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