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The protective effect of Metformin/Donepezil in diabetic mice brain: evidence from bioinformatics analysis and experiments

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Abstract. – OBJECTIVE: To identify candidate differentially expressed genes (DEGs) and pathways in diabetic mice brain with metformin/donepezil, network pharmacology analysis was used and verified by experiments.

MATERIALS AND METHODS: We analyzed GSE62013 microarray datasets derived from the Gene Expression Omnibus (GEO) database for diabetic brain. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the Database. Subsequently, the protein-protein interaction network (PPI) and Cytoscape were used for visualizing the most significant module and hub genes. Metformin/donepezil were used to treat streptozotocin (STZ)-induced diabetic mice models. Blood glucose levels and Morris water maze test were measured. The apoptotic rate of diabetic brain tissue was analyzed using Annexin V/propidium iodide double staining. The levels of PI3K and AKT in the mice brain tissues were detected by Western blot.

RESULTS: DEGs included 214 up-regulated genes and 100 down-regulated genes in diabetic brain tissues of mice. The enriched GO functions were multicellular organism development, negative regulation of transcription from RNA polymerase II promoter, and extracellular region. The enriched pathways were PI3K-Akt signaling pathway, Linoleic acid metabolism and Arachidonic acid metabolism. Blood glucose levels and apoptosis were reduced in STZ-induced diabetic mice following metformin/donepezil treatment. Metformin/donepezil could reverse this neurocognitive deficiency. Protein levels of PI3K and AKT were significantly increased in STZ-induced diabetic mice.

CONCLUSIONS: Overall, we proposed that 10 genes (Cdc20, Fbxo32, Igtp, Atg7, Fbxo15, Trim37, Psmb8, Ifi47, Asb12, and Asb5) that might be novel hub genes strongly associated with diabetic mice brain. Metformin/donepezil ameliorates STZ-induced brain injury by activating the PI3K/AKT pathway and alleviating apoptosis.

Key Words:
Bioinformatics analysis, Hub genes, Diabetic brain, Metformin/donepezil, PI3K/AKT pathway.

Introduction

The number of people with diabetes will increase to 629 million in 2045[1]. There is growing evidence that diabetes causes cognitive decline and leads to dementia both in animal models and in patients with type 1 diabetes (T1DM) or type 2 diabetes (T2DM)[2-4]. The pathophysiology of cognitive impairment in diabetes is complex, but may involve impaired insulin signaling, increased inflammation and oxidative stress pathways, and defects in metabolism[5]. Identification of key pathophysiological components is critical to the development of new therapeutic approaches for cognitive deficits[5]. Although pancreatic β cell damage has been observed in T2DM patients, the molecular mechanisms remain unknown in diabetic cognitive impairment. Understanding the pathophysiology of diabetes and identifying molecular targets and pathways will help in the treatment of the disease.

In animal experiments, treatment with metformin normalized the proteome profile and expression levels of neurogenesis markers along with improvement in the spatial memory[6,7]. For clinical trials, metformin treatment improves the cognitive function in non-dementia patients with vascular cognitive abnormalities and disorder of glucose metabolism, especially the performance function[8]. In addition, metformin and donepezil combination is more effective than metformin alone and may be considered for the treatment for diabetes-related dementia[9]. But up to now, there are few studies about the mechanism of metformin/donepezil in diabetic brain. With the wide application of genome

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transcriptome analysis, a large amount of core slice data has been produced, most of which is already stored in a public database. Re-analyzing these data can provide significant clues for other researches[10]. In this study, we downloaded the original microarray datasets, GSE62013[11], from the National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database (https://www.ncbi.nlm.nih.gov/geo). Gene expression was profiled in the streptozotocin (STZ)-induced diabetics and control mice hypothalamus. We analyzed the databases of GEO and identified the differentially expressed genes (DEGs). Then, we performed Gene Ontology (GO) and pathway enrichment analysis for the screening of DEGs, and the proteinprotein interaction (PPI) network of DEGs was constructed. The hub genes and modules of DEGs were analyzed. Furthermore, the protective effect of metformin/donepezil on brain injury in diabetic mice was performed *in vivo*.

Materials and Methods

Bioinformatics Analysis Data Source

The gene expression datasets were derived from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). A total of 29356 series about diabetes were retrieved from the database. After we use "diabetes brain" as the search term, GSE62013 gene expression profile was selected[11]. It was based on GPL8321 Mouse430A_2 Affymetrix Mouse Genome 430A 2.0 Array Volcano plot was plotted by http://www.bioinformatics.com.cn, an online data analysis and visualization platform.

Data Processing of DEGs

The GEO2R online analysis tool (https://www.ncbi.nlm. nih.gov/geo/geo2r/) was used to detect the DEGs between STZ-induced diabetic and normal mice hypothalamus, and the p-value and |logFC| were calculated. Genes that met the cutoff criteria, $p<0.05$ and $|logFC|\geq 0.5$, were considered as DEGs.

GO and KEGG Pathway Analysis

To further illustrate the main biological functions of the DEGs, we performed GO and KEGG pathway analysis using web-based DAVID tool (https://david.ncifcrf.gov/). Plots were generated by http://www.bioinformatics.com.cn. GO analysis was used to descript the genes in terms of their associated Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)[12].

PPI Network Construction and Hub Gene Identification

The STRING database (http://string-db.org/) is designed to analyze the Protein-Protein Interaction (PPI) information. To evaluate the potential PPI relationship, the DEGs were mapped to the STRING database. The PPI pairs were extracted with a combined score>0.4. Subsequently, visualize the PPI network using Cytoscape software (www.cytoscape.org/). Nodes with higher degree of connectivity tend to be more essential in maintaining the stability of the entire network. The application MCODE (version 1.6.1) was used to find core node clusters, and the application cytoHubba (version 0.1) in Cytoscape was applied to select the top 10 hub nodes ranked by degree. In this study, the top ten genes were considered hub genes. The criteria for selection included: degree cut-off = 4, MCODE scores > 4, node score cut-off = 0.2, max depth = 100 and k-score = 2.

Animals Experimental Design

This study was approved by Institutional Animal Ethical Committee of Jinzhou Medical University, Jinzhou, Liaoning, P.R. China. The mice were housed at 25°C room with 12 hours of darkness and 12 hours of light. Thirty male C57BL/6 mice (7-week-old) were randomly divided into three groups: control, STZ and treatment group. STZ and metformin/donepezil treatment group received a single intraperitoneal injection of STZ (55 mg/kg) (Cat #S0130, Sigma-Aldrich, St. Louis, MO, USA)[13]. Three days later, blood glucose levels were measured as described as described[14]. Fasting blood glucose of the mice $\geq$ 11.1 $mmol \cdot L^{-1}$ indicated successful diabetes modeling. The treatment group received a 300 mg/kg dose of metformin and 1.0 mg/kg donepezil via oral gavage for 10 days after 14 days of STZ injection. Control and STZ group were fed with the same volume of vehicle via oral gavage.

Morris Water Maze Experiment

The Morris water maze experiment was used to measure cognitive impairments as described 7 and conducted in a constant room temperature swimming pool with a white inner wall which had a diameter of 120 cm, a height of 60 cm, and a data collection and analysis system. The entire pool was divided into four quadrants by four equally spaced points (indicated by different patterns). The hidden platform (10 cm in diameter and 24 cm in height) was placed 0.5 cm below the water surface. The animals had five continuous days of maze training. During the experiment day

(sixth day), the hidden platform was eliminated, and mice were swimming in the maze for 60 s. If no platform escape incubation period is found within 60 seconds, it is recorded as 60 seconds. This experiment was repeated four times a day.

Apoptosis Assessment using Annexin V-FITC with Flow Cytometry

To confirm the apoptotic brain cells with STZ, we used Annexin V-FITC staining, following the manufacturer's instructions (BD, San Diego, CA, USA). Brain hemispheres were mechanically digested using surgical scissors blade to mince tissue and were passed through a 70-μm-filter. Samples were analyzed to determine the percentage of brain cells displaying Annexin-V/PI (+/-) staining or Annexin-V/PI (+/ +) staining in the apoptosis stage by flow cytometer.

Western Blot Analysis

Protein lysates were obtained by homogenizing the pancreatic and brain tissues (brain hemispheres). Blocked membrane was incubated with 1:1000 dilution primary rabbit antibodies (p-PI3K, PI3K, p-Akt, Akt; PROTEINTECH GROUP, Inc., Rosemont, IL, USA) for 12 h at 4°C. The membrane was incubated with secondary antibody for one hour. The grey values of bands were analyzed by densitometry analysis and normalized with β-Actin.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical significance was analyzed by one-way ANOVA; The differences with $p< 0.05$ were considered significant (in animal experiment).

Results

DEGs Identification

We used GSE62013 data set for bioinformatics analysis. In GSE62013, there were a total of 17 samples. We selected 12 sample, including 6 STZ-induced diabetics and 6 control mice hypothalamus samples. The DEGs were initially screened independently from GSE62013 with the restriction of $|logFC| \geq 0.5$ and $p<0.05$. We identified 314 DEGs from dataset (GSE62013). Of the DEGs, 214 were up-regulated and 100 were down-regulated (Figure 1).

GO and KEGG Enrichment of the DEGs

Profiled DEGs were assigned to 83 GO terms, including 47 BP, 14 CC and 22 MF terms. Typical enriched GO terms are shown in Figure 2A. The GO terms of MF category were concentrated in "GTP binding, protein binding, and hydrolase activity". The highest percentages of GO terms under CC class were "extracellular region, exter-

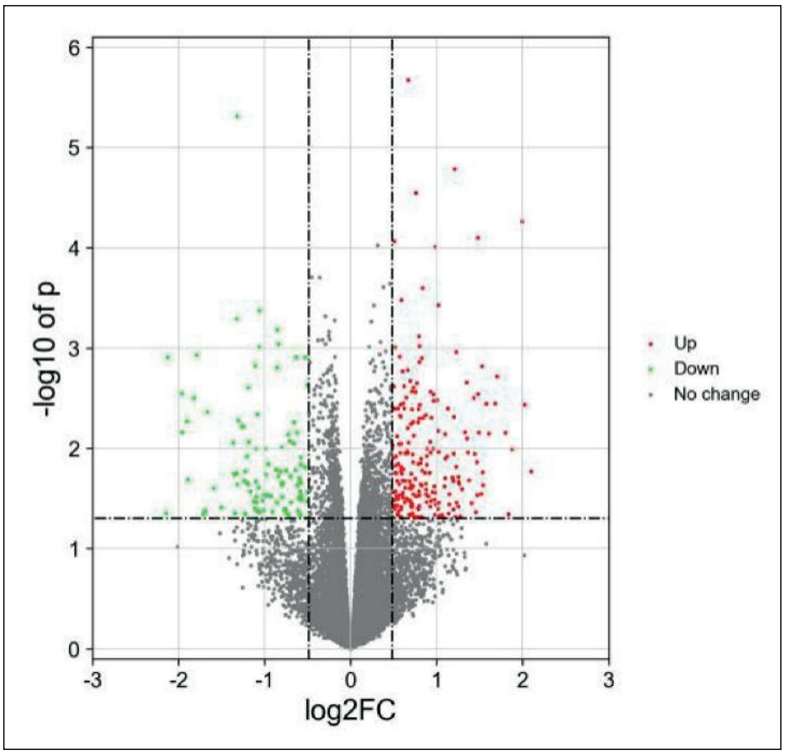

Figure 1. Identification of differentially expressed genes associated with Streptozotocin (STZ)-induced diabetics and control hypothalamus. Volcano plot for the comparison between STZ-induced diabetics and control in mice. Red color is indicative of up-regulated genes and green is indicative of down-regulated genes. The cutoff values $p<0.05$ and $|logFC|\geq 0.5$ were utilized to identify DEGs. Non-changed genes were shown in grey color.

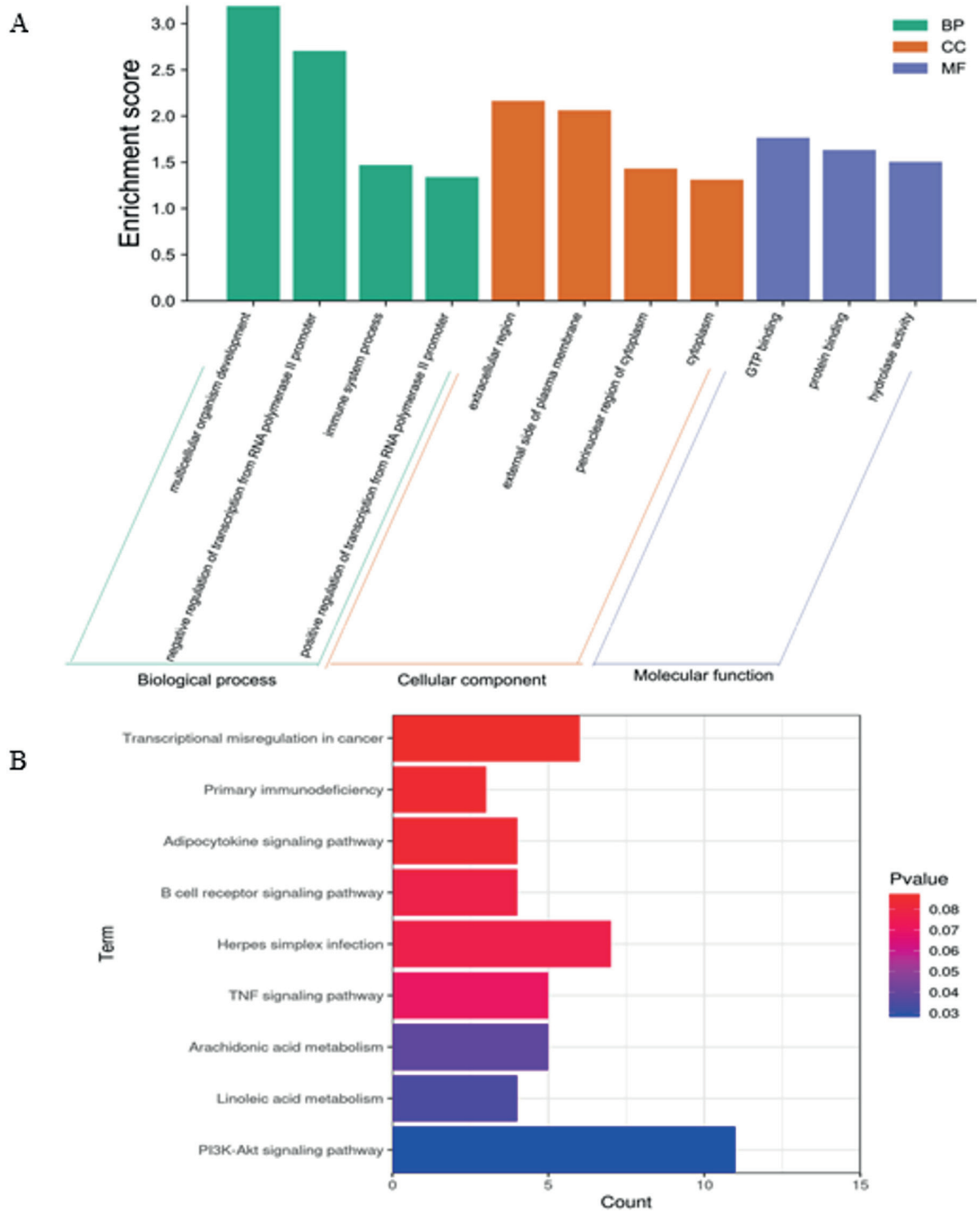

Figure 2. Functional classification of differentially expressed genes (DEGs) among different samples. **A,** GO functional classification of DEGs. The distributions are summarized in three main categories: biological process, molecular function (MF), and cellular component (CC). The x-axis indicates different GO terms, and the y-axis indicates the number of genes in each category. **B,** KEGG pathway classification of DEGs. The x-axis indicates the number of genes, and the y-axis indicates different KEGG terms in each category.

Table I. Gene Ontology (GO) function analysis using web-based DAVID tool. DEGs, differentially expressed genes.

Category	Term	Description	Count	*p*-value
GOTERM_BP_DIRECT	GO: 0007275	Multicellular organism development	28	6.47E-04
GOTERM_BP_DIRECT	GO: 0000122	Negative regulation of transcription from RNA polymerase II promoter	21	0.001976
GOTERM_BP_DIRECT	GO: 0002376	Immune system process	11	0.033927
GOTERM_BP_DIRECT	GO: 0045944	Positive regulation of transcription from RNA polymerase II promoter	21	0.045826
GOTERM_CC_DIRECT	GO: 0005576	Extracellular region	36	0.006863
GOTERM_CC_DIRECT	GO: 0009897	External side of plasma membrane	11	0.008677
GOTERM_CC_DIRECT	GO: 0048471	Perinuclear region of cytoplasm	16	0.03705
GOTERM_CC_DIRECT	GO: 0005737	Cytoplasm	100	0.048903
GOTERM_MF_DIRECT	GO: 0005525	GTP binding	12	0.017193
GOTERM_MF_DIRECT	GO: 0005515	Protein binding	71	0.023302
GOTERM_MF_DIRECT	GO: 0016787	Hydrolase activity	31	0.03134

nal side of plasma membrane, perinuclear region of cytoplasm and cytoplasm". The most prevalent "BP" terms were "multicellular organism development, negative regulation of transcription from RNA polymerase II promoter, immune system process, and positive regulation of transcription from RNA polymerase II promoter".

These results showed that most of the DEGs were significantly enriched in multicellular organism development, negative regulation of transcription from RNA polymerase II promoter, and extracellular region. The data of GO classifications are available in Table I.

For KEGG pathway evaluation, DEGs were highly clustered in several signaling pathways, such as PI3K-Akt signaling pathway, Linoleic acid metabolism and arachidonic acid metabolism (Figure 2B, Table II), suggesting that STZ may perform its function in mice hypothalamus through these pathways.

PPI Network Construction, Module and Hub Genes Analysis

A PPI network with 144 nodes and 311 edges was constructed (Figure 3A). The most significant module consisted of 20 nodes and 92 edges using the Cytoscape plugin MCODE (Figure 3B). Three significant modules from PPI networks of DEGs were extracted, including module 1 (score 9.684), module 2 (score 5), and module 3 (score 4.8). The top 10 genes (Cdc20, Fbxo32, Igtp, Atg7, Fbxo15, Trim37, Psmb8, Ifi47, Asb12, Asb5) with highest degree score were considered as hub genes (Figure 3C, Table III). More importantly, all 10 key genes were included in the module 1.

Metformin/Donepezil Ameliorated Blood Glucose in STZ-Induced Diabetes Mice Model

The results showed that fasting blood glucose was significantly increased after STZ in-

Table II. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using web-based DAVID tool. DEGs, differentially expressed genes.

Category	Term	Description	Count	*p*-value
KEGG_PATHWAY	mmu04151	PI3K-Akt signaling pathway	11	0.028218
KEGG_PATHWAY	mmu00591	Linoleic acid metabolism	4	0.034328
KEGG_PATHWAY	mmu00590	Arachidonic acid metabolism	5	0.038287
KEGG_PATHWAY	mmu04668	TNF signaling pathway	5	0.070506
KEGG_PATHWAY	mmu05168	Herpes simplex infection	7	0.077205
KEGG_PATHWAY	mmu04662	B cell receptor signaling pathway	4	0.078334
KEGG_PATHWAY	mmu04920	Adipocytokine signaling pathway	4	0.083653
KEGG_PATHWAY	mmu05340	Primary immunodeficiency	3	0.084603
KEGG_PATHWAY	mmu05202	Transcriptional misregulation in cancer	6	0.087278

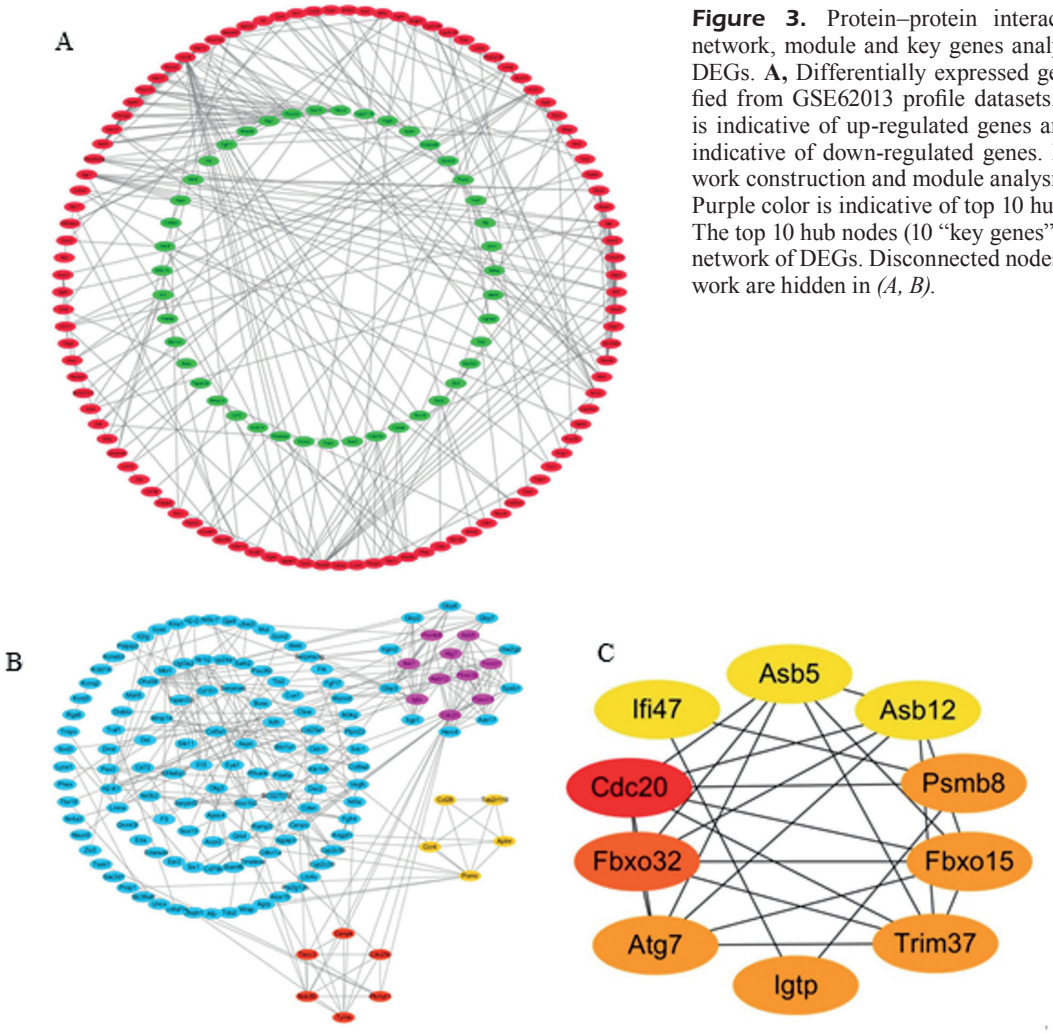

Figure 3. Protein–protein interaction (PPI) network, module and key genes analysis among DEGs. **A,** Differentially expressed genes identified from GSE62013 profile datasets. Red color is indicative of up-regulated genes and green is indicative of down-regulated genes. **B,** PPI network construction and module analysis of DEGs. Purple color is indicative of top 10 hub genes. **C.** The top 10 hub nodes (10 "key genes") in the PPI network of DEGs. Disconnected nodes in the network are hidden in *(A, B)*.

jection (Figure 4). Metformin/donepezil significantly attenuated these effects in STZ-induced mice.

Morris Water Maze

The results of the experimental day (sixth day) are displayed in Figure 5. There was a significant-

Table III. Application cytoHubba (version 0.1) in Cytoscape was applied to select the top 10 hub nodes ranked by degree.

Gene symbol	Gene Description	Degree	Up/down
Cdc20	cell division cycle 20	21	Up
Fbxo32	F-box protein 32	13	down
Igtp	interferon gamma induced GTPase	12	Up
Atg7	autophagy related 7	12	down
Fbxo15	F-box protein 15	12	Up
Trim37	tripartite motif-containing 37	12	Up
Psmb8	proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7)	12	Up
Ifi47	interferon gamma inducible protein 47	11	Up
Asb12	ankyrin repeat and SOCS box-containing 12	11	Up
Asb5	ankyrin repeat and SOCs box-containing 5	11	Up

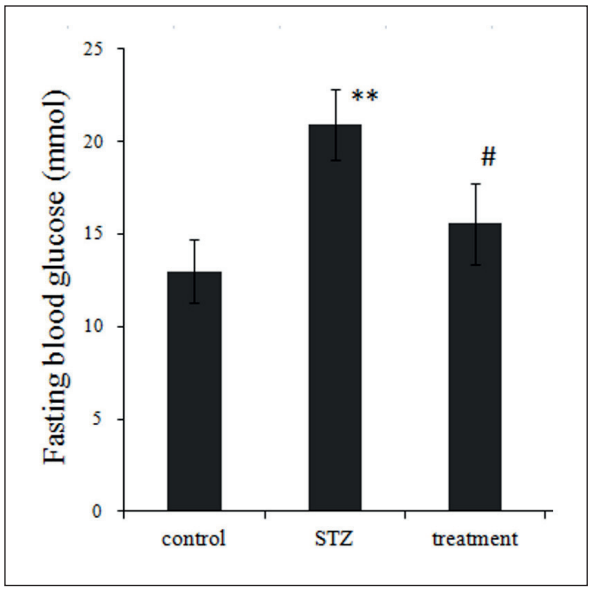

Figure 4. Effects of metformin/donepezil on fasting blood glucose levels in STZ-induced mice. **p <0.01 *vs.* control group; # p <0.05 *vs.* STZ group.

ly longer latency to escape onto the hidden platform in the STZ group compared to both the control and treatment groups at day 6. These findings indicated that STZ mice expressed poor spatial learning and memory capability and metformin/ donepezil administration could reverse this neurocognitive deficiency.

Metformin/Donepezil Decreased STZ-Induced Apoptosis of Brain

To determine the protective effect of metformin/ donepezil in STZ-induced diabetes brain, we performed flow cytometry analysis. The results exhibited that STZ-induced cell apoptosis relative to control, whereas treatment notably attenuated STZ-induced apoptosis of brain (Figure 6). These results suggested that metformin/ donepezil suppressed STZ-induced apoptosis of brain.

Metformin/Donepezil Activates the PI3K/Akt Signaling Pathway in the Brain of Diabetic Mice

Western blot analysis revealed that pAkt/Akt and pPI3K/PI3K were significantly decreased in the brain tissue of STZ group compared to control. The levels of pPI3K/PI3K and pAkt/Akt in treatment group were higher than those in STZ (Figure 7). The results indicated that diabetes inhibits the activity of PI3K and Akt, while metformin/donepezil activates the PI3K/Akt signaling pathway.

Discussion

It has been demonstrated for more than a hundred years that diabetes affects the brain. Both type 1 and type 2 diabetes are associated with mild to moderate decrements in cognitive function[15].

The cognitive impairment associated with diabetes is called “diabetic encephalopathy”. It has been coined for the patients with diabetes showing decline in their cognitive function, especially weak episodic memory, cognitive inflexibility and poor psychomotor performance leading towards Alzheimer’s disease[16]. The extensive application of gene expression profiles in diabetes raises the possibility of evaluating the pathogenesis of T2DM using bioinformatics analysis[17]. Impaired neural stem/progenitor cell proliferation is associated with decreased brain derived neurotrophic factor expression and elevated glucocorticoid levels in spontaneous or STZ-induced diabetic mice[18].

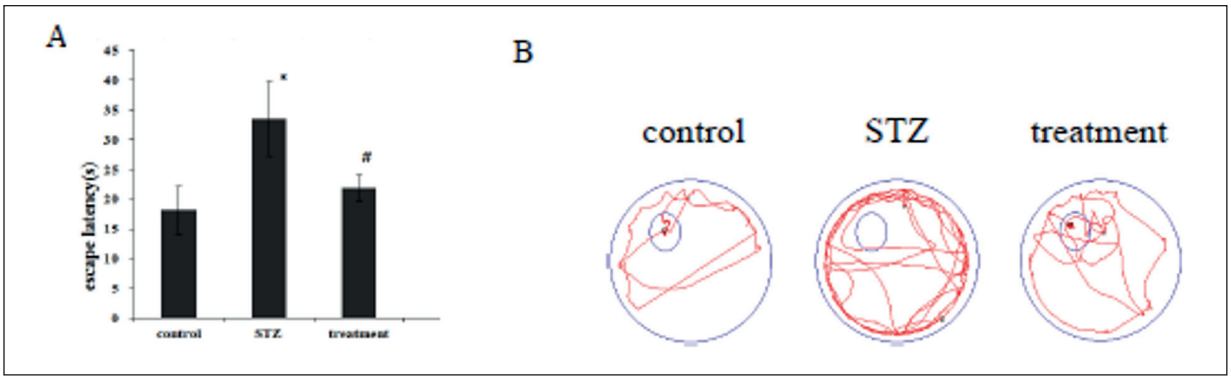

Figure 5. A, Escape latency in the Morris water maze at sixth day. *p <0.05 *vs.* control group; # p <0.05 *vs.* STZ group **B,** Representative swimming track of mice at sixth day after training.

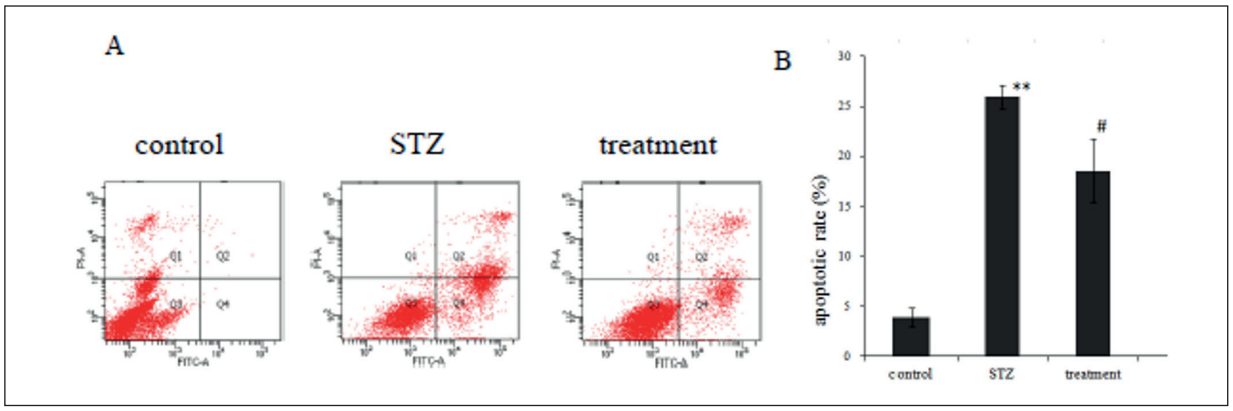

Figure 6. Effects of metformin/donepezil on apoptosis levels in STZ-induced mice. **A,** Representative images of the apoptosis analysis. **B,** Histogram representing the apoptotic rate under various conditions. $^{**}p$ <0.01 *vs.* control group; $^{\#}p$ <0.05 *vs.* STZ group.

In the present study, we analyzed the gene expression of GSE620[13] microarray data in STZ-induced diabetics brain tissue. A total of 314 DEGs were identified, including 214 upregulated genes and 100 downregulated genes. Then, we conducted in-depth studies on DEGs using bioinformatics methods, including GO and KEGG pathways, PPI networks, the most significant modules, and hub genes. Results of the Morris water maze test showed that the cognitive function of mice in the STZ group was significantly reduced, compared to the control group. Treatment with metformin/donepezil significantly improved the cognitive function of mice exposed to STZ. Metformin/donepezil inhibited brain cell apoptosis. There was a significant increase in PI3K-Akt signaling in brain of metformin/donepezil. Thus, our study suggested that metformin/donepezil might exert protective effects through relieving STZ-induced apoptosis and activating PI3K/AKT pathway.

GO enrichment analysis contains 3 groups: BP, CC, and MF. For BP, DEGs were mainly involved in multicellular organism development, negative regulation of transcription from RNA polymerase II promoter, immune system process, and positive regulation of transcription from RNA polymerase II promoter. Multicellular organisms have evolved complex strategies to preserve a relatively stable internal nutrient environment, despite fluctuations in external nutrient availability[19]. While the contributions of adaptive immunity to T1DM development are well appreciated, increasing attention has recently been given to innate immunity[20]. Insulin receptor associates with RNA polymerase II in the nucleus, with striking enrichment at promoters genome-wide. The target genes were highly

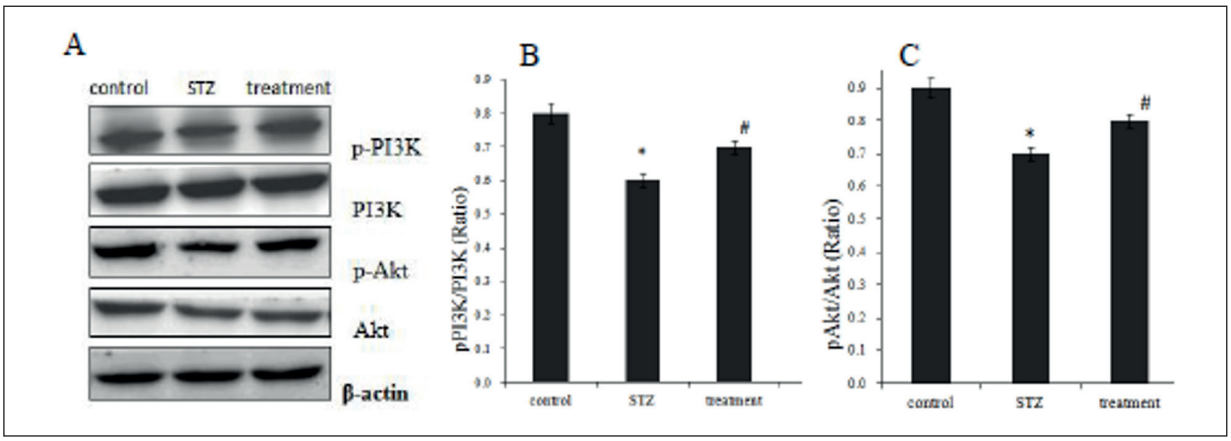

Figure 7. A, Brain PI3K/Akt signaling pathway in diabetic mice and the effects of metformin/donepezil. Metformin/donepezil significantly increase the activation of PI3K **(B)** and Akt **(C)**. $^{*}p$ <0.05 *vs.* control group; # p <0.05 *vs.* STZ group.

enriched for insulin-related functions including lipid metabolism and protein synthesis and diseases including diabetes, neurodegeneration, and cancer[21]. For CC, DEGs were mainly enriched in extracellular region, external side of plasma membrane, perinuclear region of cytoplasm, and cytoplasm[22,23]. For MF, DEGs were mainly enriched in GTP binding, protein binding, and hydrolase activity. These results suggested that the aforementioned GO terms are potentially important events in STZ-induced diabetes.

KEGG pathway analysis showed the top differentially pathways are PI3K-Akt signaling pathway, Linoleic acid metabolism and Arachidonic acid metabolism. Previous studies have demonstrated that PI3K/AKT signal pathway plays an important role in cellular physiology by mediating growth factor signals during organismal growth and critical cellular processes, such as glucose homeostasis, lipid metabolism, protein synthesis and cell proliferation and survival[24-26]. Suppression of neuronal PI3K/Akt/mTOR pathway occurs in STZ-induced type 2 DM. It may play a role in the pathobiology of hyperglycemia and neurodegeneration and dementia seen in type 2 DM [27]. These results suggested that the PI3K/AKT signal pathway are potentially important events in DM. Linoleic acid and arachidonic acid (AA) have been shown to have beneficial on glucose metabolism[28] or anti-inflammatory effects[29,30]. Thus, Linoleic acid and AA play an anti-diabetic role. In short, these studies are consistent with the results of our KEGG enrichment analysis, suggesting that PI3K-Akt signaling pathway, Linoleic acid metabolism and AA metabolism might be associated with diabetes.

In the present study, the top ten hub genes (Cdc20, Fbxo32, Igtp, Atg7, Fbxo15, Trim37, Psmb8, Ifi47, Asb12, and Asb5) were screened according to the PPI network constructed in Cytoscape by degree. Cdc20 was screened one of the top ten hub genes and might be valuable biomarkers in gestational diabetes mellitus[31], which consistent with the present study. As for Cdc20, more literature studies are about cancer. Previous studies have found that Cdc20 was closely related to apoptosis, inhibit autophagy[32-35]. In the literatures, Cdc20 plays a different role in apoptosis[32-35]. Bioinformatics studies in this paper showed that Cdc20 expression was upregulated in the diabetic group compared with the control group, but animal experimental results showed that apoptosis increased in the diabetic group. Therefore, the expression of Cdc20 in diabetes needs further study, and its effect on apoptosis remains to be confirmed.

F-box protein 32 (FBXO-32, also called atrogin-1), was significantly up-regulated in diabetic animal cardiac muscle[36,37]. The changing trend is not consistent with the biological information research results in this paper. Bioinformatics research showed that the expression of FBXO-32 was down-regulated in the hypothalamus of diabetic animals. It has been shown that interferon-r (IFN-r) is an important component of T helper cell 1 immune responses. IFN-r-induced GTPase (IGTP) has been found to be essential for host resistance to acute infections[38], and IGTP induces the activation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and promotes bacterial invasion into trophoblast giant cells[39]. A growing body of evidence implicates requisite roles for GTP and its binding proteins (Rho GTPases) in the cascade of events leading to physiological insulin secretion from the islet beta cell[40]. These results suggested that IGTP was associated with infection and DM.

Diabetes mellitus is closely related to autophagy, apoptosis and inflammation[41]. Autophagy is also implicated in neuro-protection and of interest to many biomedical researchers. Marker of autophagy (ATG7) in the hippocampus was elevated in db/db mice[42]. This is contrary to the results of this paper. This study showed that ATG7 gene was decreased in diabetic mice. We speculate that it might be because of the different models. FBXO15 (also called Fbx15) is a component of F-box proteins. Some studies have shown that F-box protein plays an important role in regulating inflammation[43]. TRIM37 plays an important role in the development of metabolic diseases[44,45]. PSMB8 may increase the CD8+ T cell proportions[46]. Inhibition of the immuno-proteasome subunit Psmb8 aggravated cytokine-induced human beta-cell apoptosis[47]. Asb5 (ankyrin repeated and Soc box-containing protein 5) may be a component for myogenesis and arteriogenesis[48]. Although little is known about the functional significance of ASB12 gene, it is postulated that the ASB family may be involved in degradation of specific proteins involved in cell proliferation or signal transduction[49]. These studies suggested that FBXO15, TRIM37, Psmb8, Asb5 and ASB12 might be associated with diabetes. At present, there are few studies on FBXO15, and further studies are needed.

Conclusions

The present study performed integrated bioinformatics analysis by using microarray datasets of diabetes brain. A total of 314 DEGs and 10 hub genes were identified. A variety of new genes and pathways might be involved in the pathogenesis of

diabetes brain. We also conclude that metformin/donepezil might exert protective effects through relieving STZ-induced apoptosis and activating PI3K/AKT pathway.

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Conflict of Interest

The Authors declare that they have no conflict of interest to declare.

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