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Screening of Potential Genes and Transcription Factors of Postoperative Cognitive Dysfunction via Bioinformatics Methods

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Background: The aim of this study was to explore the potential genes and transcription factors involved in postoperative cognitive dysfunction (POCD) via bioinformatics analysis.

Material/Methods: GSE95070 miRNA expression profiles were downloaded from Gene Expression Omnibus database, which included five hippocampal tissues from POCD mice and controls. Moreover, the differentially expressed miRNAs (DEMs) between the two groups were identified. In addition, the target genes of DEMs were predicted using Targetscan 7.1, followed by protein-protein interaction (PPI) network construction, functional enrichment analysis, pathway analysis, and prediction of transcription factors (TFs) targeting the potential targets.

Results: A total of eight DEMs were obtained, and 823 target genes were predicted, including 170 POCD-associated genes. Furthermore, potential key genes in the network were remarkably enriched in focal adhesion, protein digestion and absorption, ECM-receptor interaction, and Wnt and MAPK signaling pathways.

Conclusions: Most potential target genes were involved in the regulation of TFs, including LEF1, SP1, and AP4, which may exert strong impact on the development of POCD.

MeSH Keywords: Biological Ontologies • Cognitive Dissonance • MicroRNAs • Transcription, Genetic

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Background

Postoperative cognitive dysfunction (POCD), characterized by acute or persistent deficit or disorder in attention, memory, concentration, or learning, is a common complication following a surgical procedure that has been shown to have increasing morbidity and even mortality rates worldwide, especially among the elderly. These changes in a patient might occur only in the acute phase, or persist for days or even months to years following a surgical event, potentially leading to prolonged hospital stay, higher hospital expenses, and poor prognosis as well as deteriorated psychological status. Anesthetics such as propofol, sevoflurane, and isoflurane that are extensively used in surgery, have also been shown to be leading factors for the occurrence of POCD [1,2]. Most studies suggest that the elderly have a higher risk of POCD compared to younger patients both after anesthesia and after surgery [3,4]. Nowadays, the elderly population is increasing, especially in China, and the overall life expectancy has increased in most countries. Meanwhile, with declining physical function, the elderly present with a variety of chronic or acute diseases that need surgeries or increasing require use of anesthesia. Various studies have suggested potential associations between the occurrence of POCD and both surgery and anesthesia, yet why these correlations exist and how the internal mechanisms involved operates still requires further exploration. Increased attention has been paid to POCD research and its pathogenesis in clinical practice [5,6]. More evidence is accumulating that suggests that neuroinflammation is involved in the pathogenesis of POCD [7]. However, the precise underlying mechanism of POCD remains unclear despite great progress made in previous clinical studies. Therefore, there is an urgent need to further explore its mechanism, and search for new and potential therapeutic targets to delay or ameliorate POCD. Moreover, early identification of potential biomarkers and their molecular mechanisms in POCD continues to be debated and requires further exploration and research.

MicroRNAs (miRNAs), a class of heterogeneous non-coding small molecule RNA with the length of about 18–24 bp, play a vital role in the genesis and development of various diseases. Accumulating evidence has demonstrated that miRNAs play a critical role in the development of POCD [7,8], and multiple genes, miRNAs, and cellular pathways have been reported to contribute to the genesis and development of POCD [9,10]. Furthermore, miRNAs are involved in the pathogenesis of neurodegenerative diseases, which may also affect POCD. Cognitive function decline post-surgery, accompanied by downregulated miR-572 expression, can regulate NCAM1 expression in hippocampal neurons, and may facilitate the restoration of cognitive function *in vivo* [11]. Several potential biomarkers, such as PSD95 and NR2B, have been substantiated to be involved in the genesis and development of POCD using a bioinformatics approach [12]. Consequently, reliable findings based on online bioinformatics analysis might be useful to shed light on the mechanism of POCD.

Data mining approaches have been increasingly applied in the past decades by bioinformatics methods for high-throughput data from various microarray platforms. It has been shown that such approaches are reliable, highly accurate, and efficient in mining pathogenic biomarkers and therapeutic targets, and can assist in discovering candidate biomarkers at the molecular or cellular level [13–15]. Protein-protein interaction (PPI) network analysis is a useful tool and an efficient method for many biological processes including cell proliferation, growth, and apoptosis. It has been used for seeking key genes, and has been used to verify that protein expression is a dynamic process as demonstrated by their functions in regulating network [16–18]. Thus, many studies have investigated the molecular mechanism of POCD in the last decade to further explore the genesis and development of POCD [19,20]. In our current study, the published microarray data on miRNA expression profiles were re-analyzed. Meanwhile, differentially-expressed miRNAs (DEMs) between POCD and normal control samples were identified using bioinformatics analysis. Moreover, target genes were retrieved from these DEMs, and PPI network construction as well as pathway enrichment analysis were carried out to screen for potential biomarkers of POCD. Finally, transcription factors (TFs) targeting potential target genes were discovered. Hopefully, bioinformatics analysis can contribute to disclosing the mechanism of POCD, which can shed light on the therapeutic targets for future studies.

Material and Methods

Microarray data

The GSE95070 miRNA microarray dataset was retrieved and downloaded from a public functional genomics data repository GEO (Gene Expression Omnibus, *http://www.ncbi.nlm.nih.gov/geo/*). Microarray data were obtained based on the GPL19117 Affymetrix Multispecies miRNA-4 Array Platform (Affymetrix, Inc., Santa Clara, CA, USA), including five hippocampal tissues from POCD mice and control mice, respectively. Hippocampal tissue samples from the POCD and the control mice were enrolled in our investigation, aiming to explore the abnormal transcription of POCD.

Data processing

GEO2R on-line tool (*http://www.ncbi.nlm.nih.gov/geo/geo2r/*), which was based on limma R packages from Bioconductor [21], was used to analyze the DEMs between the control group and the POCD group. In the current study, DEMs from hippocampal tissue samples from the POCD mice and the control mice

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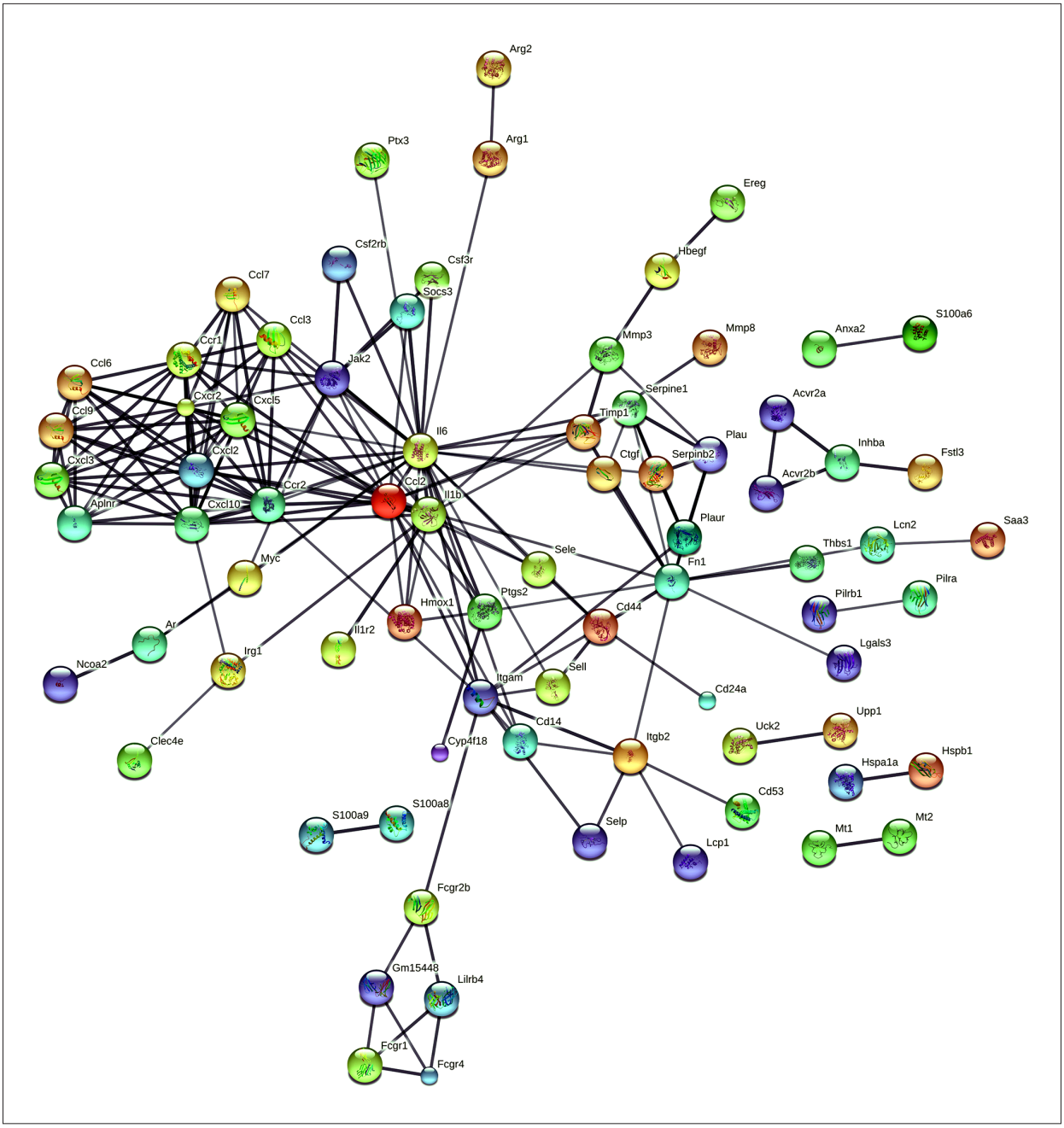

Figure 1. Protein–protein interaction network of target genes constructed by STRING 10.5.

were screened. Only miRNAs with the FDR-value of <0.05 and the $|\log_2 FC$ (fold change)| of ≥1.0 were considered as DEMs.

Prediction of miRNA targets

DEMs target genes from GSE95070 were predicted using Targetscan 7.1 (*http://www.targetscan.org*) [22,23], an online database for predicting miRNA targets. Notably, a prediction context score of -0.5 was selected as a criterion for potential target genes of each miRNA, which were identified through further analysis.

PPI network construction

STRING database (*http://string-db.org/*) [24–26] is an online software aiming to provide a critical assessment and integration of PPI, including direct (physical) and indirect (functional) associations deriving from computational prediction, knowledge transfer between organisms, and interactions aggregated from other (primary) databases. A PPI network was conducted using STRING 10.5 (*http://string-db.org/*) to analyze the correlation of potential target genes [25]. The confidence

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Table 1. The top 10 enriched GO terms in BP categories.

GO term	P value	Genes
GO: 0030199 collagen fibril organization	2.16E-09	**Col1a1**, Lox, **Col3a1**, **Col2a1**, Adamts2, **Col11a1**
GO: 0007165 signal transduction	5.55E-09	Drd5, Shh, Plcb1, F2rl2, Glp1r, Sav1, Wnt8b, Fgl2, **Gnai3**, Ect2, Olfr1029, Fzd7, Gna12, Il1r1, Gnb5, Traf3, Tacr2, Rasa1, Prokr2, Nod2, Stk3, Hif1a, Rasa4, Gnaz, Gna14, Npffr1, Rala, **Egfr**, Srgap1, Mc3r, Htr2a
GO: 0007188 G-protein signaling, coupled to cAMP nucleotide second messenger	2.28E-07	Prkacb, Gna12, Gnaz, Gna14, Mc3r
GO: 0008284 positive regulation of cell proliferation	4.16E-07	Shh, Glp1r, Fgf9, Nod2, Hif1a, Suz12, F2, Ube2a, Flt4, **Egfr**, Htr2a
GO: 0006184 GTP catabolic process	2.15E-06	**Rac1**, **Gnai3**, Gna12, Gnaz, Gna14, Rala, Tubg2
GO: 0010468 regulation of gene expression	3.25E-06	Shh, **Ppp2cb**, **Col2a1**, Hif1a, Pth, Rarg
GO: 0015031 protein transport	1.03E-05	**Col1a1**, Arfgap2, Eps15, Arf4, Ect2, Ykt6, Snx2, Rab1, Snx9, Ap3s1, **Vamp3**
GO: 0043547 positive regulation of GTPase activity	1.25E-05	Arfgap2, Plcb1, Rasa1, Snx9, Rasa4, Srgap1, Agfg1
GO: 0007049cell cycle	2.40E-05	Pak4, Stag2, **Gnai3**, Ect2, Ccnt1, Tfdp1, Rala, Csnk2a2, Pafah1b1, Pds5b, Csnk1a1
GO: 0051301cell division	4.33E-05	Stag2, **Gnai3**, Ect2, Ccnt1, Rala, Pafah1b1, Pds5b, Csnk1a1

score of >0.9 was used as the threshold. The node degree of ≥10 was set as the cutoff criterion to screen the hub genes.

Functional enrichment analysis of pathways and transcription factors of POCD

Gene Ontology (GO) annotations, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and potential transcription factors prediction were performed using GeneCodis3 web tool (*http://genecodis.cnb.csic.es/*) to identify the potential target genes [27,28]. GeneCodis3 web tool is an online tool which integrates biological information from various sources to screen their biological annotations that frequently co-occur in a series of genes. Subsequently, it ranks them based on the Benjamini and Hochberg false discovery rate method, with the p value of < 0.05 being considered as the cutoff criterion.

Results

Identification of DEMs and potential target genes

A total of three upregulated DEMs (miR-183-5p, miR-182-5p, and miR-16-1-3p) and five downregulated DEMs (miR-136-3p, miR-9, miR-592-3p, miR-29b, and miR-285-3p) were obtained from the microarray dataset GSE 95070, with the thresholds FDR of <0.05 and $|\log_2 FC|$ of ≥1.0. In addition, miR-285-3p was the miRNA with the most significant difference in POCD. Furthermore, the potential target genes predicted by Targetscan 7.1 were identified. Finally, a total of 823 potential target genes were obtained.

PPI network construction

Using the STRING online tool, a total of 308 interaction pairs were found existing among 170 proteins (genes) with the combined score of >0.9. Among them, 13 met the criteria of hub genes (Col1a1, Col11a1, Col2a1, Col3a1, Col5a3, Col7a1, Egfr, Gnai3, Kras, Ppp2ca, Ppp2cb, Rac1, and Vamp3) as shown in Figure 1. Moreover, the top three hub nodes with the highest connectivity degree were Egfr (degree=15), Ppp2cb (degree=14), and Rac1 (degree=15).

Functional and pathway enrichment analysis

GO functional analysis of potential target genes revealed 118 functional GO molecular function-associated categories and 460 biological process-associated categories. Moreover, the top 10 categories are shown in Tables 1 and 2, respectively. A total of 69 signaling pathways were identified (p<0.05), among which the top 10 most markedly enriched ones were selected according to

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Table 2. The top 10 enriched GO terms in MF categories.

GO term	P value	Genes
GO: 0005515 protein binding	1.24E-26	**Col1a1**, Eln, Peli1, Shh, Plcb1, **Rac1**, Lox, Iqcb1, Kifap3, Eps15, Brwd1, Cd274, Aicda, **Ppp2ca**, **Gnai3**, Lck, Pfn2, Ect2, Cd40, Cttn, Dmd, Rag2, Fzd7, Kif3a, Il1r1, Gnb5, Traf3, Kcnj2, Pdcd1, Ccnt1, **Ppp2cb**, Cacna2d1, Rasa1, Snx9, Nod2, POU2F1, Efna2, Cacnb4, Rpl5, Hif1a, Ndnl2, Ldlrap1, Suz12, F2, Tfdp1, Frs2, Rala, Csnk2a2, Srsf2, Ube2a, **Vamp3**, Pafah1b1, Rarg, Flt4, Crk, **Egfr**, Srgap1, Map2k6, P4ha2, Mc3r, Cxcl3, Dnmt3a
GO: 0000166 nucleotide binding	6.70E-12	Pak4, Prkacb, **Rac1**, Adh1, **Gnai3**, Adcy1, Lck, Arf4, Myo10, Kif3a, Gna12, Itpa, Rhobtb3, Gk2, Rab1, Nod2, Hnrnpf, Stk3, Adcy6, Gnaz, Eif5, Gna14, Rala, Actc1, Csnk2a2, Srsf2, Tubg2, Ube2a, Flt4, **Egfr**, Csnk1a1, Map2k6, Nsdhl, Kif3c
GO: 0005201 extracellular matrix structural constituent	8.46E-12	**Col1a1**, Col4a1, Eln, **Col3a1**, Col4a2, **Col2a1**, **Col11a1**
GO: 0005102 receptor binding	7.32E-08	Wnt8b, Fgl2, Pgr, H2-M3, Gip, Rasa1, Ldlrap1, Adcy6, F2, **Egfr**
GO: 0005525 GTP binding	3.40E-07	**Rac1**, **Gnai3**, Arf4, Gna12, Rhobtb3, Rab1, Gnaz, Eif5, Gna14, Rala, Tubg2
GO: 0046872 metal ion binding	4.31E-07	B4galt4, Znrf2, Arfgap2, Lox, Adh1, Snrnp48, Pgr, Aox4, Aicda, **Ppp2ca**, **Gnai3**, Adcy1, Dmd, Rag2, Gna12, Traf3, **Ppp2cb**, Cacna2d1, Itpa, Gm5819, Stk3, Rasa4, Adcy6, Suz12, Gnaz, Slu7, Gna14, Adamts2, Rarg, P4ha2, Agfg1, Dnmt3a
GO: 0001948 glycoprotein binding	1.74E-06	Shh, Lck, Rasa1, **Egfr**, Csnk1a1
GO: 0019901 protein kinase binding	3.53E-06	**Rac1**, Lck, Traf3, Tnni3, Ccnt1, Nod2, Hif1a, **Egfr**, Map2k6
GO: 0003924 GTPase activity	8.16E-06	**Rac1**, **Gnai3**, Gna12, Gnaz, Gna14, Rala, Tubg2

their *p* values (Table 3). Altogether, 197 TFs regulating potential target genes were identified, and the top 10 are shown in Table 4.

Discussion

MiRNAs suppress the translation of target gene transcripts and are known to have great influence on regulating gene expression at post-transcriptional levels under physiological and pathological conditions. In the current study, eight POCD-associated DEMs were screened out (namely, miR-183-5p, miR-182-5p, miR-16-1-3p, miR-136-3p, miR-9, miR-592-3p, miR-29b, and miR-285-3p) from the GEO GSE95070 dataset. A total of 823 potential target genes were obtained to identify the function of DEMs. Meanwhile, the PPI network analysis revealed that Col1a1, Col11a1, Col2a1, Col3a1, Col5a3, Col7a1, Egfr, Gnai3, Kras, Ppp2ca, Ppp2cb, Rac1, and Vamp3 were the hub nodes in the constructed PPI network. Finally, functional annotations, pathway analysis, and TFs identification were performed to determine the biological mechanisms contributing to POCD, which might provide suitable therapeutic targets for POCD.

PPI network analysis suggested that the hub genes were remarkably enriched in collagen fibril organization, signal transduction, catabolic process, as well as protein, nucleotide and GTP binding. In addition, the KEGG pathway analysis indicated that the potential target genes might participate in focal adhesion, protein digestion and absorption, ECM-receptor interaction, and Wnt and MAPK signaling pathways. Our study results indicated that many hub target genes that are involved in the GO terms and KEGG pathways, were shared by miR-182-5p, miR-183-5p, miR-29b, and miR-9, and highly correlated with POCD. Moreover, LEF1, SP1, and AP4 were also involved in the potential hub target genes regulation.

Specificity protein 1 (SP-1) is a transcription factor that plays a key role in initiating the transcription machinery to induce expression of regeneration-associated genes. SP-1 is reported to respond to inflammatory signals in the brain of Alzheimer disease (AD) patients [29]. Additionally, its expression is suggested to be upregulated in the brain of AD patients (by several folds) and in the cortex and hippocampus of transgenic AD model mice [30]. Sp5, the SP1-related transcription factor, mediates the downstream responses to Wnt/beta-catenin signaling by repressing SP-1 target genes in zebrafish in several developmental processes, including mesoderm and neuroectoderm patterning [31,32]. Expression of the typically ubiquitous SP-1 is severely restricted within forebrain neurons,

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Table 3. The top 10 enriched KEGG pathways.

KEGG pathways	P value	Genes
Kegg: 05146 Amoebiasis	3.68E-15	**Col11a1**, Col4a1, Col4a2, **Col2a1**, Gna14, Prkacb, **Col5a3**, **Col3a1**, Adcy1, Col4a5, Il1r1, Plcb1, **Col1a1**
Kegg: 04510 Focal adhesion	2.73E-13	**Col11a1**, Col4a1, Col4a2, **Col2a1**, Flt4, **Egfr**, **Col5a3**, **Col3a1**, Col4a5, Col6a2, Crk, Pak4, **Col1a1**, **Rac1**
Kegg: 04974 Protein digestion and absorption	1.80E-12	**Col11a1**, Eln, Col4a1, Col4a2, **Col2a1**, **Col5a3**, **Col3a1**, Col4a5, Col6a2, **Col1a1**
Kegg: 04512 ECM-receptor interaction	1.55E-10	**Col11a1**, Col4a1, Col4a2, **Col2a1**, **Col5a3**, **Col3a1**, Col4a5, Col6a2, **Col1a1**
Kegg: 04310 Wnt signaling pathway	1.28E-09	**Ppp2ca**, Csnk1a1, Wnt8b, Csnk2a2, Prkacb, **Ppp2cb**, Frat2, Fzd7, Plcb1, **Rac1**
Kegg: 05200 Pathways in cancer	1.89E-09	Rala, Shh, Fgf9, Col4a1, Col4a2, Traf3, Wnt8b, Hif1a, **Egfr**, Col4a5, Crk, Fzd7, **Rac1**
Kegg: 04010 MAPK signaling pathway	2.13E-09	Cacnb4, Gna12, Fgf9, Map2k6, Prkacb, **Egfr**, Il1r1, Stk3, Crk, Rasa1, **Rac1**, Cacna2d1
Kegg: 05414 Dilated cardiomyopathy	5.19E-09	Cacnb4, Dmd, Prkacb, Adcy6, Actc1, Adcy1, Tnni3, Cacna2d1
Kegg: 04062 Chemokine signaling pathway	9.20E-08	Gng5, Prkacb, Adcy6, Gnb5, Adcy1, Crk, **Gnai3**, Plcb1, **Rac1**
Kegg: 04540 Gap junction	1.05E-07	Htr2a, Prkacb, Adcy6, **Egfr**, Adcy1, **Gnai3**, Plcb1

which indicates that SP-1 might be involved in neuronal differentiation and potential dedifferentiation during degenerative processes [33]. In addition, miR-29b is also reported to inhibit fibroblast proliferation and reduce collagen deposition in rabbits subjected to glaucoma filtering surgery by restraining the PI3K/Akt/SP1 pathway [34,35]. Therefore, SP1 might participate in the neurodegeneration in optimizing neuroprotection of some nervous system diseases, indicating that SP1 might contribute to the pathogenesis of POCD.

LEF1 is significantly expressed in brain tissue, which changes throughout the embryonic and postnatal development processes [36], and it might be involved in neurogenesis and neuronal differentiation *in vivo* [37,38]. In addition, LEF1 is also reported to regulate neuron transcription through Wnt receptors [39,40]. Activating enhancer binding protein 4 (AP4), a transcription factor selectively expressed in the brain, is considered to be involved in transcriptional repression in AD [41,42]. In the current study, the potential hub genes of POCD showed a close association with the positive regulation of cell proliferation and interleukin-12 production, signal transduction, collagen fibril organization, and protein heterotrimerization. Collagen is the main component of connective tissue, which can provide support for neuronal adhesion, proliferation, and differentiation, thus repairing the injured central nervous system of adults. Collagen matrix can reduce contusion volume, neuronal loss, and cognitive deficit after traumatic brain injury, indicating that semisynthetic collagen matrix may show neuroprotection in the case of traumatic brain injury [43,44]. Some collagens have been shown in studies to be associated with the genesis and development of central nervous system diseases. Therefore, these TFs may be the key regulators in POCD development, which supports that the method adopted in the current study is effective in identifying key TFs. Critically, our findings from the PPI network analysis and key TFs prediction reveal that signal transduction of POCD is mainly related to the aforementioned hub genes. Results of key TFs predictions suggest that LEF1 and AP4 might play pivotal roles in regulating the potential key miRNAs contributing to POCD, such as miR-183-5p and miR-9.

Conclusions

In the current study, a number of DEMs were identified in POCD based on bioinformatics analysis. The results of the target gene prediction and the PPI network construction suggested that Col1a1, Col11a1, Col2a1, Col3a1, Col5a3, Col7a1, Egfr, Gnai3, Kras, Ppp2ca, Ppp2cb, Rac1, Vamp3 and POCD displayed strong correlations. Moreover, the results of GO enrichment, KEGG signal pathway analysis, and target genes regulated by TFs further confirmed that LEF1, SP1, and AP4 were potential key TFs, and might be associated with the pathogenesis of POCD. All these conclusions provide new insights

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Table 4. The top 10 TFs targeting more than five genes.

TF	P value	Genes
SP1	5.36E-13	POU2F1, Ap3s1, Gna12, Ube2a, Eln, Kcnj2, **Col2a1**, Cdh3, Pafah1b1, Stag2, H2afz, Rarg, Snx2, Dnmt3a, Prkacb, **Col5a3**, Lrat, Eps15, Adamts2, Kif3a, Col4a5, **Col7a1**, Frs2, Pdcd1, Kifap3, Crk, Fzd7, Rasa1, Pgr, Rpl36al, Plcb1, Rasa4, Pak4, Eif5, Iqcb1, **Col1a1**
LEF1	1.55E-11	Myo10, Col11a1, Shh, Gna12, Ube2a, Fgf9, Psme4, Gcnt2, Map2k6, Kcnj2, **Col2a1**, Cdh3, Wnt8b, Stag2, Dmd, Rarg, Ccnt1, Dnmt3a, F2, **Col5a3**, **Col3a1**, Lrat, B3galt2, Kif3c, Kif3a, Lox, Fzd7, Pfn2, Pak4, Eif5, Rhobtb3, **Col1a1**, Cacna2d1
MAZ	1.98E-09	POU2F1, Ap3s1, Col11a1, Shh, Aicda, Fgf9, Map2k6, Pafah1b1, Stag2, H2afz, Rpl5, Dmd, Rarg, Lck, Galm, Dnmt3a, F2, **Col5a3**, Col4a5, Arf4, Stk3, Tnni3, Kifap3, Fzd7, Pgr, Rasa4, **Col1a1**
AP4	8.28E-09	Aicda, Eln, Mybl2, Map2k6, Kcnj2, Traf3, **Col2a1**, Cdh3, Wnt8b, Hif1a, H2afz, Dmd, Lck, Srgap1, Col5a3, B3galt2, Adamts2, **Col7a1**, Arf4, **Vamp3**, Tubg2
PAX4	6.70E-08	POU2F1, Ap3s1, Shh, Ube2a, Eln, Pafah1b1, Stag2, Hif1a, Rarg, Lck, Folr1, **Col5a3**, Kif3c, Crk, Fzd7, Rasa1, Plcb1, **Col1a1**, **Rac1**
TAL1ALPHAE47	7.95E-08	Ap3s1, Fgf9, Rag2, Kcnj2, Stag2, Dmd, B3galt2, Stk3, Plcb1
AREB6	2.33E-07	Shh, Aicda, Fgf9, Kcnj2, **Col2a1**, Stag2, Hif1a, Dmd, Dnmt3a, Prkacb, Srgap1, Hebp1, Rpl36al, Plcb1, Eif5
HSF1	2.77E-07	Fgf9, Wnt8b, Stag2, H2afz, Flt4, Dmd, Cdh10, Adcy6, Adamts2, Col4a5, Eif5
MYOD	6.37E-07	POU2F1, Aicda, Kcnj2, H2afz, Csnk2a2, Dmd, Cdh10, Dnmt3a, Adcy6, Lrat, B3galt2, **Col7a1**, Hebp1, Stk3, Plcb1
RP58	7.64E-07	Fgf9, Rag2, Dmd, Rarg, **Col3a1**, B3galt2, Adamts2, **Col7a1**
TAL1BETAITF2	1.09E-06	Fgf9, Rag2, Kcnj2, Stag2, Dmd, B3galt2, Stk3, Plcb1
PAX2	1.72E-06	Ube2a, Fgf9, Pafah1b1, Dmd, **Egfr**, Lox, Drd5, Eif5
CHX10	2.28E-06	POU2F1, **Ppp2ca**, Wnt8b, Stag2, H2afz, Dmd, Folr1, Cdh10, Col4a5, Stk3, Fzd7, Pgr, **Col1a1**
FOXO4	4.44E-06	POU2F1, Gng5, Fgf9, Psme4, Map2k6, Stag2, Hif1a, Dmd, Cdh10, B3galt2, Col4a5, Frs2, Lox, Znrf2, Fzd7, Plcb1, Iqcb1, **Col1a1**, Cacna2d1, Ndnl2
E12	6.52E-06	POU2F1, Shh, Gna12, Fgf9, Eln, Kcnj2, **Col2a1**, Cdh3, Nck2, Hif1a, Gna14, H2afz, Dmd, Rarg, Dnmt3a, Srgap1, Lrat, Kif3c, Stk3, Drd5, Plcb1, **Col1a1**

into the roles of critical TFs in POCD rat models, and supports our hypothesis that the collagen family might have a key impact on regulating the potential key TFs including LEF1, SP1, and AP4 in POCD. However, no other studies at present have shown that these TFs are linked with POCD. Therefore, further studies regarding the association of these potential biomarkers with POCD are required to further prove the results obtained in our research.

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