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Identification of neuron-related genes for cell therapy of neurological disorders by network analysis*#

Li-ning SU, Xiao-qing SONG, Hui-ping WEI†‡, Hai-feng YIN
(*Department of Biology, Hebei North University, Zhangjiakou 075029, China*)
†E-mail: whp123456@sina.com

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Abstract: Bone mesenchymal stem cells (BMSCs) differentiated into neurons have been widely proposed for use in cell therapy of many neurological disorders. It is therefore important to understand the molecular mechanisms underlying this differentiation. We screened differentially expressed genes between immature neural tissues and untreated BMSCs to identify the genes responsible for neuronal differentiation from BMSCs. GSE68243 gene microarray data of rat BMSCs and GSE18860 gene microarray data of rat neurons were received from the Gene Expression Omnibus database. Transcriptome Analysis Console software showed that 1248 genes were up-regulated and 1273 were down-regulated in neurons compared with BMSCs. Gene Ontology functional enrichment, protein-protein interaction networks, functional modules, and hub genes were analyzed using DAVID, STRING 10, BiNGO tool, and Network Analyzer software, revealing that nine hub genes, *Nrcam*, *Sema3a*, *Mapk8*, *Dlg4*, *Slit1*, *Creb1*, *Ntrk2*, *Cntn2*, and *Pax6*, may play a pivotal role in neuronal differentiation from BMSCs. Seven genes, *Dcx*, *Nrcam*, *Sema3a*, *Cntn2*, *Slit1*, *Ephb1*, and *Pax6*, were shown to be hub nodes within the neuronal development network, while six genes, *Fgf2*, *Tgfβ1*, *Vegfa*, *Serpine1*, *Il6*, and *Stat1*, appeared to play an important role in suppressing neuronal differentiation. However, additional studies are required to confirm these results.

Key words: Neuronal differentiation; Bone mesenchymal stem cells (BMSCs); Protein-protein interaction network; Differentially expressed genes
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1 Introduction

Neurological disorders are problems of the nervous system, which may be possible to treat by the transplantation and differentiation of stem cells to enhance nerve regeneration. Several studies have shown that bone mesenchymal stem cells (BMSCs) can be induced and differentiated into neural cells under specific, controlled microenvironments (Jiang *et al*., 2005; Çapkın *et al*., 2012). Thus, BMSCs are known as "seed cells" in the cell therapy of many neurological disorders and have the advantage of being easy to isolate.

Many factors have been reported to induce neuronal cell differentiation from BMSCs, including basic fibroblast growth factor, epidermal growth factor, neurotrophic factors, vascular endothelial growth factor, hepatocyte growth factor, β-mercaptoethanol, dimethylsulfoxide, and butylated hydroxyanisole (Bae *et al*., 2011; Edamura *et al*., 2014; Parivar *et al*., 2015). Bertani *et al*. (2005) previously showed that gene expression was altered in BMSCs exposed to butylated hydroxyanisole and dimethylsulfoxide neural induction medium, but did not match the set of genes differentially expressed in immature neural tissues compared with untreated BMSCs. However,

‡ Corresponding author
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ORCID: Li-ning SU, http://orcid.org/0000-0001-8118-7601
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because of the many factors involved with neuronal cell differentiation, including risk, survival time, and instability, the use of BMSCs has limited application in the clinic. In particular, the process of BMSC differentiation into nerve cells is unstable, and so it is important to define more stable differentiation conditions.

In this study, microarray data were examined to identify genes expressed differentially between immature neural tissues and BMSCs. Bioinformatic methods were used to analyze Gene Ontology (GO) functional enrichment and to construct protein-protein interaction (PPI) networks. We also identified high connectivity (hub genes) associated with neuronal differentiation and neuronal development.

2 Materials and methods

2.1 Materials

The gene expression profiles of GSE68243 and GSE18860 were obtained from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo). Dataset GSE68243 includes three untreated Sprague-Dawley rat BMSC samples (GSM1666210, GSM1666211, and GSM1666212), while dataset GSE18860 includes three cortical neuron samples from d18 Sprague-Dawley rat embryos (GSM4674922, GSM4674923, and GSM4674924). Both datasets are based on GPL6247 platform information.

2.2 Data preprocessing

The data downloaded as CEL files were preprocessed using Expression Console™ software and underwent background correction and quartile data normalization by the robust multiarray average algorithm (Irizarry *et al*., 2003) with default parameters. Non-annotated probes were filtered out.

2.3 Screening of differentially expressed genes and hierarchical cluster analysis

Differentially expressed genes (DEGs) between neurons and BMSCs were identified by one-way between-subjects analysis of variance based on Transcriptome Analysis Console software (Torres *et al*., 2015). Genes with fold-changes $|\log_2|>4$ were selected as DEGs, and P of <0.05 and false discovery rate (FDR) of <0.05 were used as thresholds.

2.4 Functional enrichment analysis

GO is widely used in functional studies and the enrichment analysis of gene sets. The DAVID (Sherman *et al*., 2007) gene annotation tool was used to analyze GO enrichment in DEG functions. Values of $P<0.05$ and FDR<0.05 were again used as cutoff criteria. GO terms were displayed as a significant network using the BiNGO Plug-in of Cytoscape v. 3.2.1 software (Shannon *et al*., 2003). DEGs associated with neural differentiation were clustered in neuronal differentiation and regulation of cell proliferation modules.

2.5 Construction of GO:0030182, GO:0048666, and GO:0042127 PPI networks, and the identification of hub genes

The STRING 10 database (Snel *et al*., 2000) provides known and predicted interactions based on confidence. The interactions contain direct (physical) and indirect (functional) associations, which are derived from genomic context, experiments, coexpression, and previous knowledge. Genes in the neuronal differentiation and regulation of cell proliferation modules were considered to be seed nodes and were mapped onto the STRING database to construct extended PPI networks with medium confidence scores of 0.4. All PPI networks were visualized by Cytoscape v. 3.2.1 software and calculated using the Network Analyzer tool (Assenov *et al*., 2008) based on parameters of betweenness centrality (BC) and degree. BC reflects the number of shortest paths that pass one node, and is essential in the analysis of nodal importance. The degree indicates the number of interactions of a particular protein. In the final networks, nodes with a high degree were displayed within a large circle while shades of green and yellow represented high to low BC values for the node (Assenov *et al*., 2008). In this study, nodes with thresholds of BC>0.05 and degrees above the average value of each network were considered to be hub genes.

3 Results

3.1 DEG screening

The standardization of expression data revealed a good degree based on all the black lines in Fig. 1 on the same straight line. A total of 2521 genes were

identified as being differentially expressed between neurons and BMSCs, 1248 of which were up-regulated and 1273 down-regulated in neurons compared with BMSCs (Table S1). Hierarchical cluster analysis showed that the three neuronal samples were distributed within the neuronal sample cluster and that the three BMSC samples were within the BMSC sample cluster (Fig. 2). This showed that grouping was rational and that the data could be used directly for further analysis.

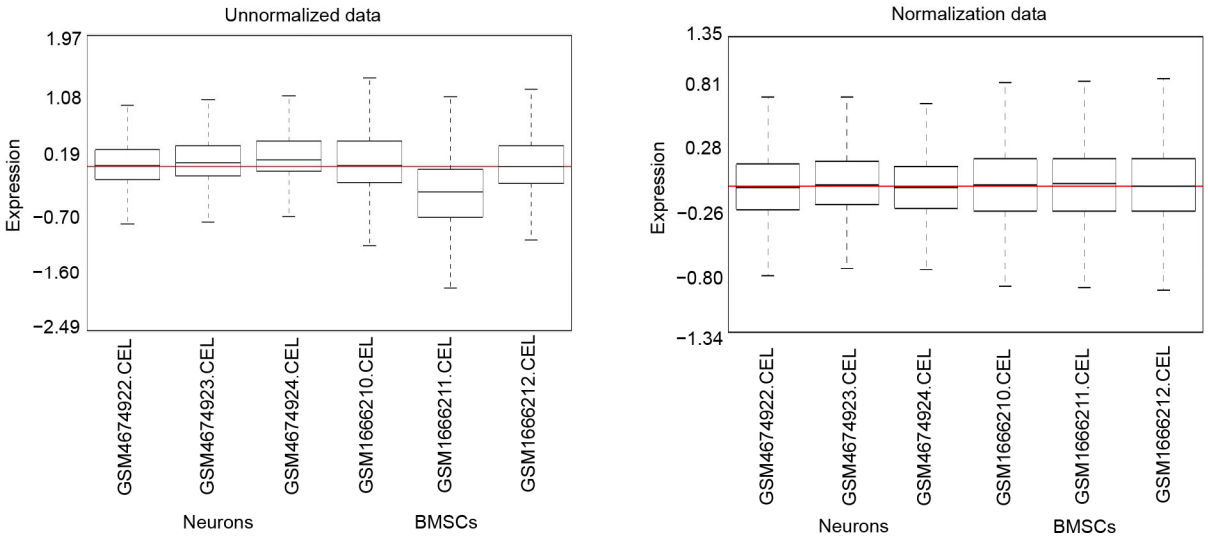

Fig. 1 Cassette figures of the expression data before and after standardization
The horizontal axis represents sample names while the vertical axis means the expression value

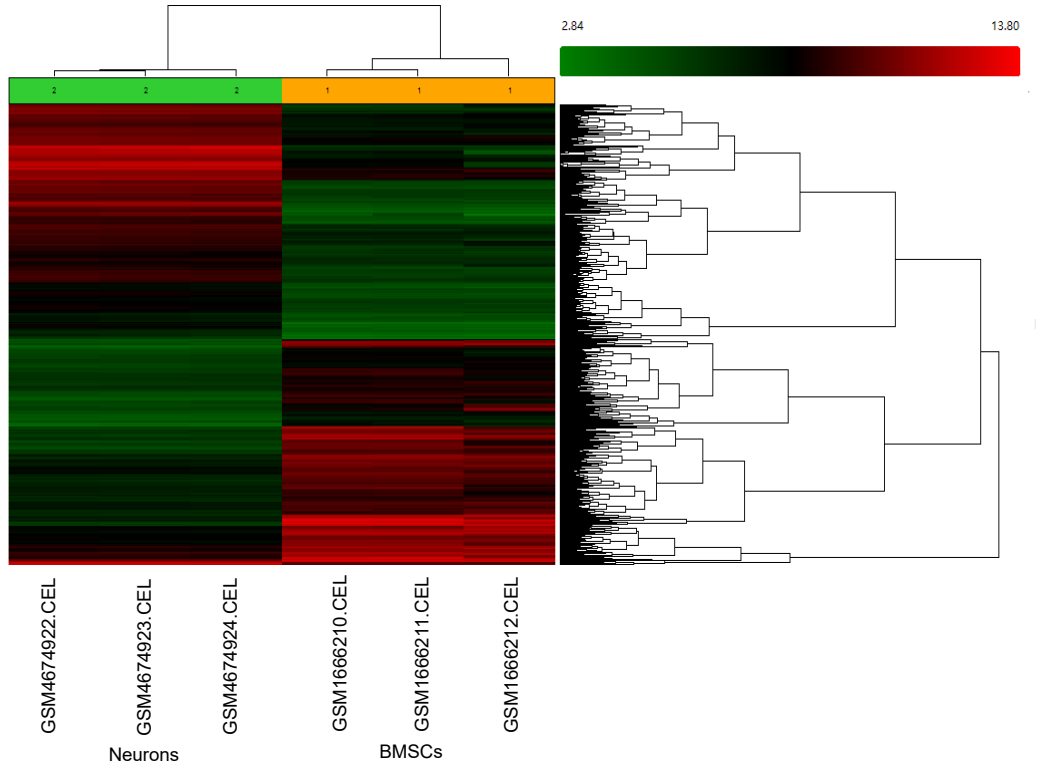

Fig. 2 Hierarchical cluster analysis of DEGs
The horizontal axis shows sample names. GSM1666210, GSM1666211, and GSM1666212 are BMSC samples. GSM4674922, GSM4674923, and GSM4674924 are neuron samples. The right vertical axis represents clusters of DEGs, and the above horizontal axis means clusters of samples. Red means up-regulated genes and green signifies down-regulated genes

3.2 Functional enrichment analysis

GO enrichment analyzed using DAVID software revealed that the up-regulated genes were significantly enriched in the biological processes of neuronal differentiation (GO:0030182), neuronal development (GO:0048666), transmission of nerve impulses (GO:0019226), synaptic transmission (GO:0007268), and neuron projection development (GO:0031175) (Table S2, Fig. 3a), while down-regulated genes were significantly enriched in regulation of cell proliferation (GO:0042127), response to organic substances (GO:0010033), wound healing (GO:0042060), response to wounding (GO:0009611), and blood vessel development (GO:0001568) (Table S2, Fig. 3b). GO terms for up-regulated and down-regulated genes were calculated using the BiNGO Plug-in of Cytoscape software, and significant terms shown using yellow nodes (Fig. 4).

3.3 PPI network analysis of GO:0030182, GO:0048666, and GO:0042127

To investigate the molecular mechanism of neural differentiation from BMSCs, we selected the up-regulated genes involved in neuronal differentiation (GO:0030182) and neuronal development (GO:0048666), and the down-regulated genes involved in regulation of cell proliferation (GO:0042127) because these are closely associated with neuronal cell differentiation. Genes of the three GO terms (Table 1) were used to construct PPI networks with the STRING database. Networks were visualized and analyzed using Cytoscape v. 3.2.1 software. Analysis of nodal properties in each network revealed average degrees of 6.39 for GO:0030182 nodes, 4.88 for GO:0048666 nodes, and 11.94 for GO:0042127 nodes. A total of nine genes were identified as hub genes for neuronal differentiation: neuronal cell

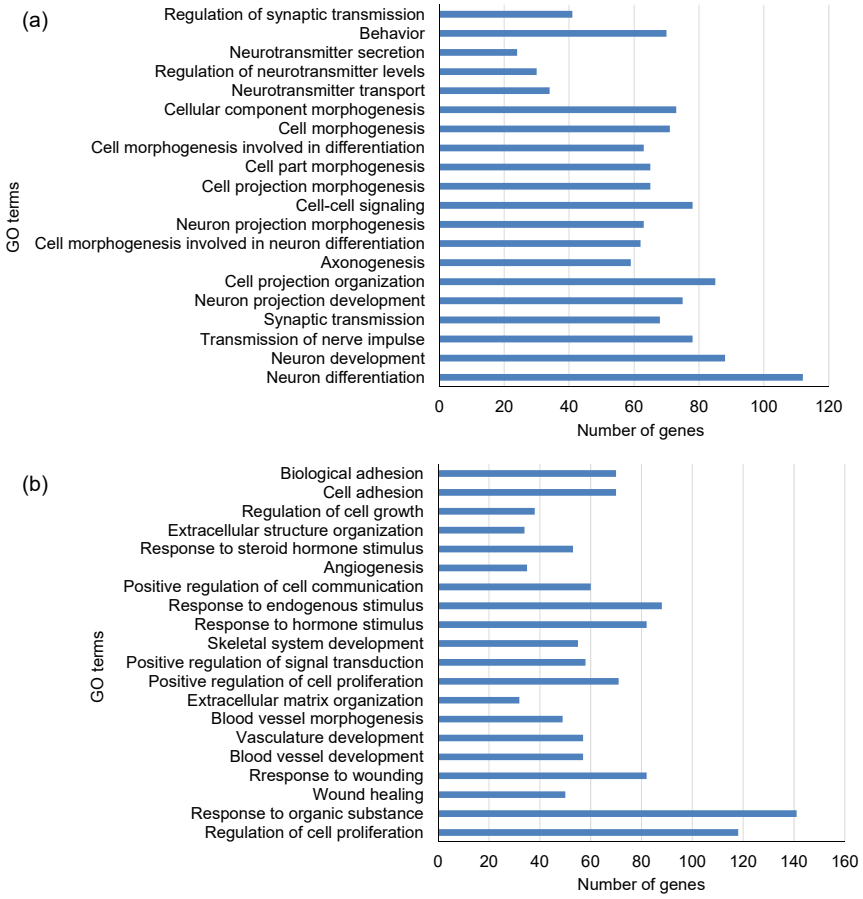

Fig. 3 GO enrichment analyses

(a) The top 20 GO enrichment terms of up-regulated genes of neurons compared with BMSCs; (b) The top 20 GO enrichment terms of down-regulated genes of neurons compared with BMSCs. The horizontal axis represents the number of DEGs. The vertical axis represents the enriched GO terms. *P* values increase from bottom to top

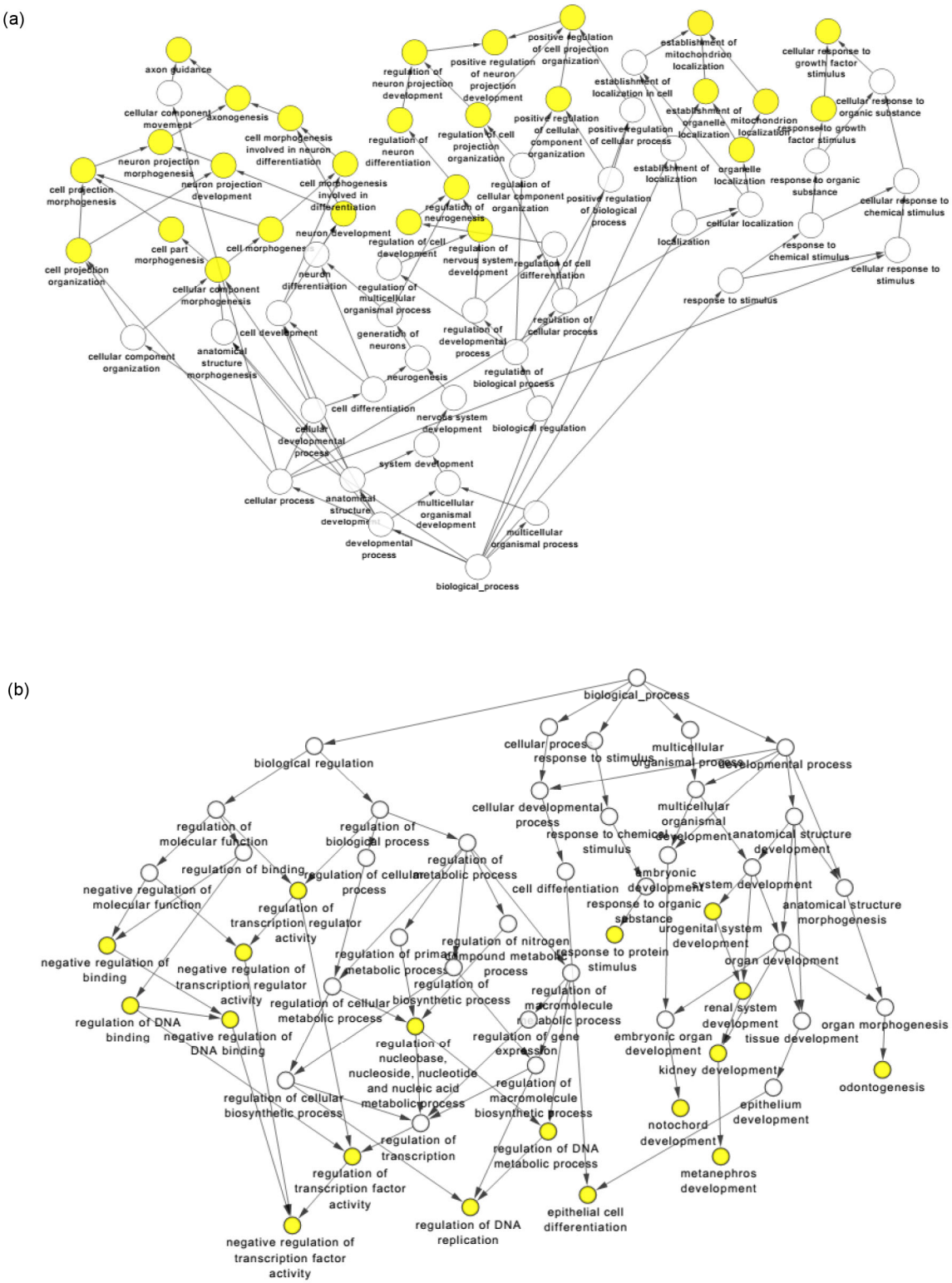

Fig. 4 GO terms displayed as a network using BiNGO

(a) Up-regulated genes GO terms; (b) Down-regulated genes GO terms. Yellow nodes: nodes with *P*-value<0.05 and FDR<0.05

adhesion molecule (*Nrcam*), semaphoring-3A (*Sema3a*), mitogen-activated protein kinase 8 (*Mapk8*), discs large MAGUK scaffold protein 4 (*Dlg4*), slit homolog 1 (*Slit1*), cyclic adenosine monophosphate (cAMP)-responsive element binding protein 1 (*Creb1*), neurotrophic tyrosine kinase receptor 2 (*Ntrk2*), contactin 2 (*Cntn2*), and paired box gene 6 (*Pax6*); seven genes were identified as hub genes for neuronal development: doublecortin (*Dcx*), *Nrcam*, *Sema3a*, *Cntn2*, *Slit1*, ephrin type-B receptor 1 (*Ephb1*), and *Pax6*; and six genes were identified as hub genes for the regulation of cell proliferation: basic fibroblast growth factor 2 (*Fgf2*), transforming growth factor β-1 (*Tgfβ1*), vascular endothelial growth factor A (*Vegfa*), plasminogen activator inhibitor-1 (*Serpine1*), interleukin 6 (*Il6*), and signal transducer and activator of transcription (*Stat1*) (Fig. 5 and Table 2).

4 Discussion

Neuronal differentiation from BMSCs offers a number of advantages for cell therapy of neurological disorders, but an understanding of the underlying cellular and genetic mechanisms is required. Through the comparative analyses of gene expression data in neuronal cells and BMSCs, we identified a total of 2521 DEGs between the two cell types, of which 1248 were up-regulated and 1273 were down-regulated in neuronal cells compared with BMSCs (Table S1). GO functional enrichment analysis showed that of the up-regulated genes, 10.46% were associated with the neuronal differentiation GO term, 8.22% with the neuronal development GO term, and 7.28% with the transmission of nerve impulses. Among the down-regulated genes, GO functions were associated with the regulation of cell proliferation, which is related to

Table 1 Genes in GO terms of neuron differentiation and regulation of cell proliferation

GO terms	Biological process	Genes
GO:0030182	Neuron differentiation	*Gprin1*, *Plxna3*, *Efna2*, *Rorb*, *I1cam*, *Cspg5*, *Klhl1*, *Nrcam*, *Atp2b2*, *Ank3*, *Unc5a*, *Robo1*, *Ss18l1*, *Dscam*, *Stmn3*, *Emx1*, *Efnb3*, *Mdga1*, *Mdga2*, *Kif5c*, *Dll1*, *Tbr1*, *Ctnna2*, *Slitrk2*, *Ascl1*, *Slitrk1*, *Ncam2*, *Slitrk3*, *Foxg1*, *Reln*, *Mapk8*, *Stmn1*, *Igsf9*, *Slitrk5*, *Cdk5r1*, *Lppr4*, *Sox2*, *Sox5*, *Rgd1311558*, *Ephb1*, *Ephb2*, *Arx*, *Crmp1*, *Lhx2*, *Lhx6*, *Olfm3*, *Bhlhe22*, *Celsr3*, *Evl*, *Celsr2*, *Gas7*, *Dlx2*, *Fezf2*, *Epha4*, *Epha7*, *Nrep*, *Dlx1*, *Sema6c*, *Ntrk2*, *Map2*, *Chrnb2*, *Syngap1*, *Atl1*, *Agtpbp1*, *Uchl1*, *Pax6*, *Vgf*, *Gpc2*, *Casp3*, *Dynll2*, *Mapt*, *Dlg4*, *Sema3a*, *Dlg2*, *Tubb3*, *Kcnma1*, *Slit1*, *Psd*, *Sema4f*, *Cntn2*, *Cntn4*, *Gap43*, *Dcc*, *Rab3a*, *Cck*, *Ndn*, *Nnat*, *Brsk1*, *Bcl11b*, *Pou3f4*, *Pou3f2*, *Cd24*, *Dcx*, *Snap25*, *Nefl*, *Dclk1*, *Dfna5*, *Gnao1*, *Ptprz1*, *Creb1*, *Nfasc*, *Dpysl5*, *Nlgn1*, *Ntng2*, *Dpysl4*, *Isl1*, *Wnt7b*, *Ppp1r9a*, *Neurod2*,*Wnt7a*, *Apbb1*, *Fez1*
GO:0048666	Neuron development	*Gprin1*, *Plxna3*, *Atl1*, *Efna2*, *Uchl1*, *Pax6*, *I1cam*, *Rorb*, *Cspg5*, *Klhl1*, *Nrcam*, *Atp2b2*, *Ank3*, *Unc5a*, *Dynll2*, *Robo1*, *Dlg4*, *Sema3a*, *Ss18l1*, *Dlg2*, *Dscam*, *Stmn3*, *Efnb3*, *Kif5c*, *Tbr1*, *Slit1*, *Ctnna2*, *Slitrk2*, *Ncam2*, *Ascl1*, *Slitrk1*, *Slitrk3*, *Sema4f*, *Foxg1*, *Cntn2*, *Cntn4*, *Mapk8*, *Reln*, *Stmn1*, *Igsf9*, *Slitrk5*, *Gap43*, *Dcc*, *Rab3a*, *Cdk5r1*, *Lppr4*, *Cck*, *Ndn*, *Rgd1311558*, *Ephb1*, *Ephb2*, *Arx*, *Crmp1*, *Bcl11b*, *Lhx2*, *Lhx6*, *Cd24*, *Dcx*, *Olfm3*, *Snap25*, *Nefl*, *Dclk1*, *Gnao1*, *Ptprz1*, *Creb1*, *Nfasc*, *Nlgn1*, *Ntng2*, *Celsr3*, *Dpysl4*, *Celsr2*, *Evl*, *Isl1*, *Gas7*, *Fezf2*, *Epha4*, *Epha7*, *Nrep*, *Wnt7b*, *Ppp1r9a*, *Sema6c*, *Ntrk2*, *Map2*, *Neurod2*, *Chrnb2*, *Syngap1*, *Apbb1*, *Fez1*
GO:0042127	Regulation of cell proliferation	*Dlc1*, *Apobec1*, *Thrb*, *Ptgs2*, *Il6st*, *Osmr*, *Pgf*, *Pdgfa*, *Ptgs1*, *Fgfrl1*, *Tgfβ3*, *Fgf10*, *Tlr4*, *Jag1*, *Pmaip1*, *Il15*, *Cxcl12*, *Tgfβ1*, *Tgfβ2*, *H19*, *Pgr*, *Myd88*, *S1pr1*, *Cdkn2b*, *Serpine1*, *Pdgfc*, *Rarg*, *Mfge8*, *Thy1*, *Vegfc*, *Cd38*, *Serpinf1*, *Ptprv*, *F3*, *Vegfa*, *Pdgfra*, *Wfdc1*, *Tgif1*, *Ripk2*, *Pdgfrb*, *Pmp22*, *Ngf*, *Cav2*, *Cav1*, *Ccl2*, *Ifitm3*, *Ifi30*, *Itgb1*, *Irak4*, *Lif*, *Vdr*, *Itgav*, *Runx2*, *Runx3*, *Bmp4*, *B4galt1*, *Bmp2*, *Sphk1*, *Tgfbr2*, *Hgf*, *Shox2*, *Notch2*, *Pla2g4a*, *Cdkn1a*, *Atf3*, *Cd274*, *Avpr1a*, *Kctd11*, *Klf4*, *Plau*, *Sat1*, *Ppard*, *Fgf7*, *Acvrl1*, *Pparg*, *Prrx1*, *Gja1*, *Pawr*, *Prrx2*, *Tenc1*, *Ddr2*, *Wisp2*, *Ang*, *Hmox1*, *Hey2*, *Shc1*, *Fgf1*, *Il13ra1*, *Fgf2*, *Fosl1*, *Lyn*, *Cdk6*, *Mbd2*, *Mmp12*, *Pura*, *Cth*, *Ccnd1*, *Adam17*, *Tbx18*, *Eif5a2*, *Csf1*, *Nr3c1*, *Stat6*, *Camk2d*, *Plcd1*, *Cd28*, *Nox4*, *Ptprc*, *Il6*, *Tbx3*, *Tbx2*, *Anxa1*, *Sparc*, *Stat1*, *Clec11a*, *Cyba*, *Nupr1*, *Id3*

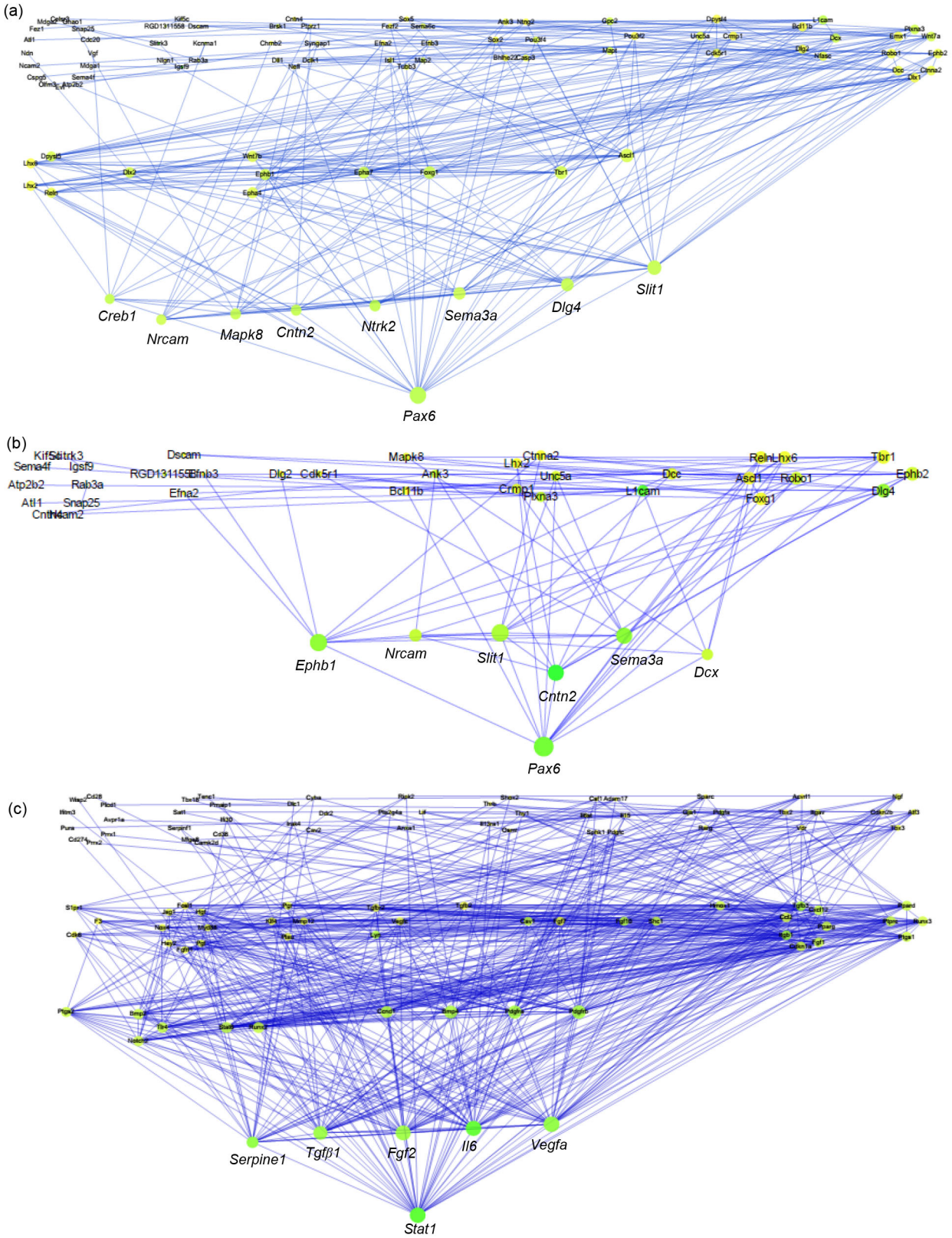

Fig. 5 PPI networks of neuron differentiation (GO:0030182, a), neuron development (GO:0048666, b), and regulation of cell proliferation (GO:0042127, c) biological process

Node color: shades of green to yellow color depict node with highest to lowest values of betweenness centrality (BC); Node size: sizes from biggest to smallest circle mean the node degrees. Bigger and dark colored nodes represent genes with more links

Table 2 Key genes selected based on visualize parameters like BC and degree

Network name	Gene	Degree	BC	AD
Neuron differentiation	*Nrcam*	11	0.052 742 72	6.39
	Sema3a	15	0.053 825 15	
	Mapk8	12	0.090 814 13	
	Dlg4	16	0.103 825 38	
	Slit1	19	0.108 426 54	
	Cntn2	12	0.110 418 82	
	Creb1	10	0.113 116 98	
	Ntrk2	13	0.122 851 31	
	Pax6	24	0.163 991 00	
Neuron development	*Dcx*	6	0.058 705 83	4.88
	Nrcam	7	0.066 951 81	
	Sema3a	10	0.145 349 80	
	Cntn2	10	0.268 125 51	
	Slit1	11	0.101 701 35	
	Ephb1	11	0.138 484 58	
	Pax6	13	0.181 375 54	
Regulation of cell proliferation	*Fgf2*	38	0.059 946 33	11.94
	Tgfβ1	34	0.063 818 16	
	Vegfa	40	0.074 373 06	
	Serpine1	26	0.074 690 11	
	Il6	39	0.116 884 44	
	Stat1	40	0.126 546 91	

BC: betweenness centrality; AD: average degree of all nodes

cell differentiation, response to organic substances, and wound healing (Table S2). Neuronal differentiation, neuronal development, and cell proliferation are closely associated with neuronal cell differentiation, indicating that the findings could be viewed with high confidence.

In the present study, specific DEGs were up-regulated in neural tissue compared with untreated BMSCs. These include *Sema3a*, *Mapk8*, *Nrcam*, *Dlg4*, *Slit1*, *Creb1*, *Ntrk2*, *Cntn2*, and *Pax6*, which play important roles in neuronal differentiation, while *Dcx*, *Nrcam*, *Sema3a*, *Cntn2*, *Slit1*, *Ephb1*, and *Pax6* may have a critical role in neuronal development. *Fgf2*, *Tgfβ1*, *Vegfa*, *Serpine1*, *Il6*, and *Stat1* were selected as key genes in the regulation of cell proliferation network, and were down-regulated in neural tissue compared with untreated BMSCs; this implies a role in suppressing neuronal differentiation. *Slit1* has previously been reported to be involved in the regeneration and functional recovery of the trigeminal ganglion and inferior alveolar nerve (Ceber *et al*., 2015), while a role for *Slit1* was identified in the development of dopaminergic neurons, which is mediated by NCK adaptor protein 2 to trigger changes in cortical neuron morphology (Round and Sun, 2011). PAX6 is a highly conserved multifunctional transcription factor, which has been demonstrated to be a key protein in neurogenesis and neuronal plasticity by influencing cascades of gene expression (Mishra *et al*., 2015). In retinoic acid-induced neuronal differentiation, PAX6 was found to be repressed by the recruitment of histone modifier lysine-specific demethylase 1 (Wu *et al*., 2016). Our results showed that *Pax6* is a key gene in neuronal differentiation and neuronal development.

CNTN2 was previously suggested to control axonal growth both in vitro and in vivo. Ratie *et al*. (2014) showed that *Cntn2* expression was restricted to the early axon scaffold populations, cranial ganglia, and spinal motor neurons, as well as mature neurons. However, we observed a likely role for *Cntn2* in neuronal differentiation and development. NRCAM, a neuronal cell adhesion molecule, is important in aspects of neural development including cell proliferation and differentiation, axonal growth and guidance, synapse formation, and formation of the myelinated nerve structure (Sakurai, 2012), which is involved in neuron-neuron and neuron-glial adhesions. NRCAM also modulates axonal growth and guidance by a mechanism involving the neuronal receptor axonin-1 (Lustig *et al*., 1999). NRCAM deletion in mice previously led to elevated spine densities on the apical dendrites of star pyramidal cells during both postnatal and adult stages. NRCAM was also found to form a complex with semaphorin 3F (Sema3F) in the brain to regulate the spine density of star pyramidal neurons (Demyanenko *et al*., 2014). *Dcx* expression was followed in migrating and differentiating neurons in the brains of embryonic mouse (Francis *et al*., 1999). In postnatal mice, reducing the levels of DCX caused abnormal neuronal migration, which affected neuronal development in the postnatal forebrain (Belvindrah *et al*., 2011). Within the two main adult neurogenic regions, *Dcx* was shown to be transiently expressed in dividing neural progenitor cells until neuronal maturation (Brown *et al*., 2003). We observed a likely role for DCX and NRCAM in neuronal development.

Sema3a is a member of the class 3 secreted semaphorin and has been shown to induce growth

cone collapse or repel sensory axons of the dorsal root ganglia in vitro (Wu *et al*., 2014) and regulate adult neurogenesis by controlling stem cells proliferation and differentiation in the subventricular zone (Sun *et al*., 2016). A recent study has shown that distinct cytoplasmic domains in Plexin-A4 mediated diverse responses to *Sema3a* in developing mammalian neurons (Mlechkovich *et al*., 2014). EPHB1 has been indicated to play important roles in synaptic plasticity of the nervous system (Liu *et al*., 2009), with *Ephb1* null mice exhibiting neuronal loss in the substantia nigra pars reticulata and spontaneous locomotor hyperactivity (Richards *et al*., 2007). Little is known about the role of *Dlg4*, *Ntrk2*, *Creb1*, or *Mapk8* in neuronal differentiation and development.

In the present study, *Stat1* was represented as the hub gene of regulation of the cell proliferation network, showing the highest degree and BC. The knockdown of *Stat1* previously demonstrated that interferon γ (IFN-γ) inhibits the differentiation of neuronal precursor cells by negatively regulating the expression of *Neurog2* via the Janus kinase/Stat pathway (Ahn *et al*., 2015). Pereira *et al*. (2015) reported that IFN-γ induced neuronal differentiation and acted as an antiproliferative factor via STAT1 in the adult subventricular zone, thereby regulating neurogenesis in normal adult brains. IL-6 has been shown to suppress neurogenesis within human retinal cell suspensions in the presence of N2 culture medium containing FGF2, which enhances neurosphere generation (Balasubramaniam *et al*., 2009). FGF2 was reported to be a potent neurotrophic factor that promotes the differentiation of BMSCs into dopaminergic neurons and enhances their survival both in vitro and in vivo (Nandy *et al*., 2014). FGF2 has also been widely used to induce neuronal differentiation in mice and primates, including humans, but was shown to promote self-renewal in a macaque embryonic stem cell line; its effects on the differentiation of stem cells appear to be influenced by the presence or absence of supplemental retinoic acid (Hatori *et al*., 2014). Additionally, FGF2 has been found to delay neurogenesis in embryonic chicks (McGowan *et al*., 2013). Moreover, low concentrations of FGF2 increased neurogenesis in human neural progenitor cells while high levels maintained progenitor cell proliferation and blocked neurogenesis (Nelson and Svendsen, 2006). This result is consistent with our own findings.

VEGF is known to modulate learning and memory, the plasticity of mature neurons, synaptic transmission, and neurogenesis (Yang *et al*., 2016). However, evidence for its role in the regulation of excitatory synaptic activity is conflicting (Yang *et al*., 2016). In our analysis, *Vegfa* appeared to be an inhibitory factor in neuronal differentiation. A previous study suggested that TGF-β1 enhances neuronal excitability (Zhang *et al*., 2016) and protects midbrain dopaminergic neurons from IFN-γ-induced neurotoxicity (Zhou *et al*., 2015), but there is no evidence to indicate that TGF-β1 suppresses neuronal differentiation. Finally, the role of *Serpine1* in neuronal cells is currently unclear.

5 Conclusions

According to the centrality-lethality rule, hubs are more likely to be functionally relevant than other genes (Nair *et al*., 2014). In this study, we identified that a number of hub genes, including *Sema3a*, *Nrcam*, *Slit1*, *Cntn2*, and *Pax6*, have positive roles in neuronal differentiation and neuronal development, that *Mapk8*, *Dlg4*, *Creb1*, and *Ntrk2* play pivotal roles in neuronal differentiation, and that *Nrcam* and *Ephb1* are important in neuronal development. Previous studies have shown that these genes have important functions in neuronal differentiation and neuronal development, with the exception of *Dlg4*, *Ntrk2*, *Creb1*, and *Mapk8*. *Fgf2*, *Tgfβ1*, *Vegfa*, *Serpine1*, *Il6*, and *Stat1* were shown to suppress neuronal differentiation, while *Vegfa* and *Tgfβ1* were documented as having positive roles in neurogenesis. Further studies are required to confirm these findings.

Compliance with ethics guidelines

Li-ning SU, Xiao-qing SONG, Hui-ping WEI, and Hai-feng YIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Information of differentially expressed genes between neurons and BMSCs

Table S2 Gene ontology enrichment analysis of biological process

中文概要

题　目：基于芯片数据的神经分化基因网络互作分析为神经系统疾病的细胞疗法提供基础

目　的：通过筛选差异基因，获得控制骨髓间充质干细胞向神经细胞分化及神经发育的中心基因，为治疗神经系统疾病提供参考。

方　法：从基因表达综合数据库（Gene Expression Omnibus database）中获得芯片数据，利用生物信息学软件筛选差异基因，并对差异基因进行 GO 功能富集、蛋白互作网络分析和中心基因分析。

结　论：通过分析，初步推测 *Nrcam*、*Sema3a*、*Mapk8*、*Dlg4*、*Slit1*、*Creb1*、*Ntrk2*、*Cntn2* 和 *Pax6* 等中心基因在调控骨髓间充质干细胞向神经细胞的分化中发挥重要作用；*Dcx*、*Nrcam*、*Sema3a*、*Cntn2*、*Slit1*、*Ephb1* 和 *Pax6* 等中心基因在神经发育过程中发挥作用；*Fgf2*、*Tgfβ1*、*Vegfa*、*Serpine1*、*Il6* 和 *Stat1* 等中心基因在抑制神经分化过程中发挥作用。

关键词：神经分化；骨髓间充质干细胞；蛋白质相互作用网络；差异基因