Bioinformatics analysis of differentially expressed genes in human embryonic stem cells and their differentiated dorsal root ganglion

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Article

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Abstract

To explore the difference of gene expression between undifferentiated hESCs and DRG induced by hESCs differentiation for 1 day by bioinformatics technology, and find out the key differential genes and their potential molecular regulation mechanism; The functions involved are analyzed and predicted. Download the gene chip data series GSE75582 related to stem cells and dorsal root ganglion from the geo expression database, import the gene chip online analysis tool GEO2R, screen out the differential genes, and construct the volcano map. Webgestalt database was used for GO function analysis and KEGG pathway enrichment analysis, and string analysis and Cytoscape software were used to construct PPI network. Results 4102 differentially expressed genes were screened, of which 2674 were up-regulated and 1428 were down regulated. Bioinformatics analysis of differentially expressed genes showed that the genes were mainly enriched in axon guidance, signaling pathways regulating pluripotency of stem cells, pathways in cancer, ECM receiver interaction, focal adhesion, cell adhesion molecules (CAMs), PI3K Akt signaling pathway, MAPK signaling pathway, TGF beta signaling pathway Ras signaling pathway, which extracts differential genes in stem cell related signaling pathways, including 34 up-regulated genes and 13 down regulated genes. Conclusion BMP4, Sox2, FGF2, Nanog, Smad3, KLF4, gsk3b and FGFR2 in undifferentiated hESCs are closely related to stem cell self-renewal; BMP4, Sox2, FGF2, Smad3, gsk3b and FGFR2 were mostly related to neuropathic pain and neurite growth. However, the functions of these key genes need to be further confirmed by future experimental studies.

Introduction

Human pluripotent stem cells (hPSCs) are divided into two cell types: human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs). The study of hESCs provides insights into human development and disease, however, establishing hESCs is difficult, especially ethical. [1]. hESCs has unlimited self-renewal and differentiation ability. It provides a powerful in vitro model for the differentiation of three germ layer derivatives of human body. It can also be used as an in vitro model to study human neurogenesis and neurodegeneration[2,3]. There is evidence that hESCs can selectively differentiate into the neural epithelium without induction and acquire neural crest (NC) capacity[4]. hESCs-derived GABA-enabling neurons can play a therapeutic role in epileptic animals by establishing inhibitory synapses with host neurons[5]. In addition, hESCs-derived oligodendrocytes are not only able to reveal the fate of oligodendrocytes, but they also have therapeutic potential in combination with the support of growth factors of axons and neurons, and can be applied as cell replacement therapy (CRT) to the study of myelin regeneration[6]. The effective in vitro neural differentiation of hESCs provides a cellular source for a wide range of molecular and cellular neuroscience research questions, from ion channel properties to axon regenerative behavior. Currently includes the production and or enrichment of stage-specific nerve cells, such as pluripotent neural epithelial cells, lineage-directed neural progenitor cells, and post-mitosis neurons and glial subtypes, which will serve as valuable tools for drug screening and cell therapy[7]. The dorsal root ganglia (DRG) of the peripheral system is often used as a model for studying neuropathic pain, and the presence of immune interactions in DRG plays a key role in the development of neuropathic

pain after nerve injury, and it is believed that its mechanism may be related to cell-to-cell communication and certain receptors[8]. In recent years, researchers have focused on DRG showing a powerful regenerative response after damage to the peripheral nervous system. Isolated rodent DRG was shown to be sensory neurons that promote somatic sensation and were used to study the growth, regeneration and degeneration of neurites and the formation of peripheral nervous system （PNS ）myelin sheaths[9]. In summary, hESCs have been reported more in the central nervous system（CNS） than PNS, and the basic mechanism of their application and differentiation in PNS is still unclear, and further detailed research is needed. In this study, the differences in gene expression between hESCs and their induced differentiation DRG were explored by bioinformatics technology, and the key differential genes and their potential molecular regulatory mechanisms were identified, and the functions involved were analyzed and predicted. Provide a reference for the potential to produce valuable and stable human nerve cell subtypes in PNS.

Materials And Methods

1. The data of this study are from the National Center of biotechnology Information, NCBI). Gene Expression Omnibus (GEO), Use the following Searches: "Stem Cells" "DRG" (keyword), "Array Expression Analysis" (Research Type), and "Tissue" (Attribute Name). The inclusion criteria were as follows: (1) human embryonic stem cells diagnosed as undifferentiated and induced human embryonic stem cells differentiated into DRG samples for 1 day, (2) the number of samples in each group was more than 4, (3) mRNA gene expression profile, (4) sufficient gene probe information for analysis. Then, the gene expression profile serial number GSE75582 was collected for analysis. The experiment used the expression profile chip of Affymetrix company of the United States (the sample data of neurons and neural stem cells have been standardized).
2. GEO2R（https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE75582 ）differentially expressed genes between embryonic stem cells and DRG were screened. Filter the gene expression deletion data according to the gene name and the gray value of gene chip signal, and perform the fold change test on the standard gene signal value (log2 FC（- 2 or log2 FC > 2, P < 0.05), that is, take the difference multiple as the test standard, take the logarithm for data conversion, use the double difference to obtain the significantly differentially expressed genes, and statistically screen them with the pvalue. Using webtest database（ http://www.webgestalt.org/ ）The differentially expressed genes were analyzed by go, and their enrichment was analyzed from the perspective of molecular function, biological process and cell composition, At the same time, KEGG analysis was carried out to enrich it from the perspective of signal pathway. Using string database（ https://cn.string-db.org/ ）The differential genes were analyzed by protein protein interactions (PPI). Analyze the PPI network diagram to discover the proteins and related proteins at the key nodes and their corresponding relationship and stability. Cytoscape software (a popular open source software tool) is used to visually explore the interaction network between biomolecules composed of proteins, genes and other types of interactions, so as to integrate gene data and visualize the up-down regulation mapping of differential genes.

Results

1. A total of 4102 genes with obvious expression differences were obtained by GEO2R analysis, an online analysis tool of gene chip, There were 2674 upregulated genes and 1428 downregulated genes in undifferentiated hESCs and hESCs differentiated into 1-day DRG The box plot indicates the degree of dispersion of the sample, and the correlation analysis generates a volcano map (**Figures 1, 2**).
2. GO analysis up-regulated genes showed that there were 1490 genes enriched in biological processes, 1186 genes enriched in cell composition, and 1411 genes enriched in molecular function **(Figure 3**). The results of the GO analysis of down-regulated genes involved showed that there were 754 genes enriched in biological processes, 606 genes enriched in cell composition, and 696 genes enriched in molecular function (**Figure 4**). KEGG results analyzed the main differences in metabolic pathways between undifferentiated hESCs and hESCs differentiated into 1-day DRG (**Figure 5, Table 1**). Extraction of stem cell-related signaling pathways from undifferentiated hESCs and hESCs differentiated into 1-day DRG differential genes (**Table 2**).
3. String analysis results of differentially expressed genes in stem cell related signal pathways PPI network analysis of differentially expressed genes through string online tool shows that there are 47 nodes (proteins corresponding to differentially expressed genes and their related proteins) and 178 edges (identification of interaction relationship between proteins corresponding to differentially expressed genes and their related proteins) (**Figure 6)**.
4. The top ten hub genes on the stem cell related signal pathway were screened by Cytoscape software (**Figure 7 Table 3**).

Discussion

Studies have shown that stem cells from different sources have the ability to form neurons and glia after transplantation, and help the regeneration of surviving sensory neurons, especially in the DRG cavity and the damaged area of DRG[10]. Sacai et al[11]showed that neuroectodermal progenitor cells in human bone marrow stromal cells (hBMSCs) can be selectively expanded and then induced to differentiate into Schwann like cells. The co-culture of Schwann like cells and embryonic DRG neurons promotes the formation of Schwann like cells, which has been proved to be related to injury repair and myelination in vivo. There are a large number of high-purity human neuronal progenitors (hNPs) in hESCs, and when hESCs are transplanted into the injured spinal cord, hNPs are naturally integrated into the host tissue and various mature neuronal subtypes are generated, and the whole process is deeply affected by the cellular microenvironment[12]. Kitazawa A's team[13]found that the combination of medium (CM) and nerve growth factor (NGF) in chicken back root ganglion (DRG) conditions had an impact on the directional differentiation of ESCs. They confirmed that DRG-CM can effectively promote ESCs differentiation into neurons. Vidal M[14]identified neural crest-derived stem cells in DRG; in vitro, these cells are able to form pluripotent spheroids that produce neurons, glial cells, and myofibroblasts, and while DRG appears to

have stem cell potential, their identity and pathophysiology remain unknown. In order to confirm whether DRG itself or after induction by stem cells has stem cell properties, researchers are still exploring.

In this study, 4102 genes with different expression between undifferentiated hESCs and DRG differentiated by hESCs for one day were screened, including 2674 up-regulated genes and 1428 down regulated genes. Go analysis showed that the enriched genes were mainly involved in biological regulation, metabolic process, cell membrane and nuclear synthesis, intimal system, regulating protein, regulating nucleic acid and other metabolic processes. KEGG analysis showed that the differential genes were mainly distributed in axon guidance, signaling pathways regulating pluripotency of stem cells, pathways in cancer, ECM receiver interaction, focal adhesion, cell adhesion molecules (CAMs), PI3K Akt signaling pathway, MAPK signaling pathway, TGF beta signaling pathway and Ras signaling pathway; In the signal pathway regulating stem cell pluripotency, 34 genes were up-regulated and 13 genes were down-regulated. Finally, differential genes analyze the signal pathway regulating stem cell pluripotency through PPI network. It finds that the core protein interaction in the up-regulated genes is stable. Based on the analysis of Cytoscape software algorithm, the common hub genes on this pathway are BMP4, SOX2, FGF2, Nanog, Smad3, KLF4, GS3B and FGFR2.

In the above hub gene, the up-regulated genes include BMP4, SOX2, FGF2, NANOG, SMAD3, KLF4, FGFR2, and the downregulated gene is GSK3B. Bone morphogenetic protein 4 (BMP4) induces hESCs to differentiate into a trophoblast layer[15]. This induction process of BMP4 is mainly achieved by inhibiting the extracellular receptor kinase (ERK) and p38 mitosogen-activated protein kinase (MAPK) pathways[16]. In DRG, BMP4 is able to inhibit synaptic growth[17]. SOX2 is a key regulator of a variety of stem cells, particularly ESCs and neural progenitor cells (NPCs). Understanding the functional mechanisms of SOX2 can help realize the potential of ESC and NPCs. SOX2 inhibits non-neural lineages in hESCs and regulates neurogenesis in hNPC by inhibiting classical Wnt signaling[18]. Cells in DRG capable of highly expressing neural crest cell markers include Nestin, Sox2, Sox10, and p75, where Sox2-positive cells, especially satellite glial cells associated with chronic pain, can induce neurogenesis[19, 20]. Fibroblast growth factor 2 (FGF2) promotes self-renewal of hESCs[21]. hESCs that overexpress FGF2 have a neuroprotective effect and can shift the body to an anti-inflammatory environment[22]. However, FGF2 inhibits the synaptic growth of dorsal root ganglion neurons (DRGN)[23]. NANOG is a key transcription factor for ESCs pluripotency. NANOG has three paralogous homologs in human cells, namely NANOG1, NANOG2 and NANOGP8, hESCs express large amounts of NANOG1 and NANO2. NanoGP8, NANOGP4 and NANOGP5 are expressed in human cancer cells. Notably, in some cancer cells, NANOGP8 produces levels of the NANOG protein comparable to those produced by NANOG1 in pluripotent cells; cancer-associated NANOGP8 helps promote dedifferentiation and cell plasticity[24]. The transcriptional regulation of NANOG itself in hESCs is largely elusive, and Chan's findings [25]show that NANOG 's two novel upstream transcriptional activators are functionally important for self-renewal of hESCs. However, NANOG coverage in DRG is almost non-existent. SMAD3 is an intracellular medium that transduces signals from transforming growth factor (TGF) and activin receptors[26]. Efficient transformation of hESCs and iPS cells in the nervous system can be achieved by inhibiting SMAD signaling[27]. The expression of the

activator downstream signaling molecule, SMAD3 mRNA, decreases with the development of DRG in chicken embryos. Elisa results showed that activin A through SMAD3 may play an important role in the early development of embryonic DRG, which is associated with inhibiting glial cell proliferation and regulating the release of the neurotransmitter GABA[28]. Krüppel-like factor 4 (KLF4) directly regulates hESCs[29], Gaining insight into the regulation of core transcription factors helps to better control the self-renewal and pluripotency of hESCs. However, the transcriptional regulation of NANOG itself in hESCs is largely elusive. Related studies have found that during the differentiation of hESCs, the mRNA and protein expressions of KLF4 and PBX1 are down-regulated. In addition, the overexpression of KLF4 and PBX1 upregulated the activity of THE NANOGO promoter in hESCs and the expression of endogenous NANOG proteins, thus affecting the self-renewal of hESCs[30]. Although several components of the fibroblast growth factor (FGF) signaling pathway have been reported to be detected in hESCs, the function of this pathway and its effect on cell fate decisions have not been determined. In Petr's study, the expression of FGF-2 and its receptors (FGFRs) in undifferentiated and differentiated hESCs was found. FGF-2 can influence hESCs as exogenous and endogenous factors. In addition, hESCs release FGF-2 into the medium, which indicates that they have autocrine activity[31]. FGFR activation in DRG was found after peripheral nerve injury (PNSI) in rats, but this activation process is mostly associated with pain[32]. Glycogen synthase kinase 3 (GSK3) is a key enzyme involved in glycogen metabolism, but is now known to regulate a variety of cellular functions[33]. In ESCs culture, certain small molecules alter key signaling pathways to promote cell self-renewal and inhibit differentiation. In mice, for example, small molecules in the FGF/MEK/Erk and GSK3B pathways inhibit the formation of the mesoderm[34]. GSK-3B inhibitor TDZD-8 has a certain effect on the axon growth of dorsal rhizobia neurons in neonatal rats, low concentration of TDZD-8 can promote axonal growth, the formation of multiple axons or axon branches, while high concentration of TDZD-8 significantly inhibits axonal growth, resulting in axonal retraction[35]. In general, reports of hub gene-related genes regulating the signaling pathways of stem cell pluripotency are more common in hESCs, most of which are transcription factors, which have clearly dominated the self-renewal and cell fate of ESCs; although transcriptional regulators have been extensively studied in hESCs, the extent of their contribution to pluripotency is still unclear[36].

Bioinformatics technology was used to analyze gene chip data of undifferentiated hESCs and DRG that induced hESCs differentiation for 1 day, and the GEO2R analysis tool, webgestalt database, STRING analysis and Cytoscape software were used to screen out the differentially expressed genes in undifferentiated hESCs and DRG that induced hESCs differentiation for 1 day, and conducted in-depth research on the relationship between biological function and protein interaction network. To explore the possible molecular mechanisms of differential genes expressed, it will help to study the clinical treatment of stem cells.

In summary, the results of this study show that there is a significant expression difference gene between undifferentiated hESCs and DRG that induces the differentiation of hESCs for 1 day, and signaling pathways that regulate stem cell pluripotency are analyzed, and it is found that BMP4, SOX2, FGF2, NANOG, SMAD3, KLF4, GSK3B, FGFR2 are closely related to stem cell self-renewal in undifferentiated

hESCs. In DRG, where hESCs differentiate for 1 day, BMP4, SOX2, FGF2, SMAD3, GSK3B, and FGFR2 are mostly related to neuropathic pain and neurite growth, and their stem cell characteristics need to be further explored.

Abbreviations

BMP4 Bone Morphogenetic Protein 4

CAMs Cell Adhesion Molecules

CRT Cell Replacement Therapy

CNS Central Nervous System

CM Combination of Medium

DRG Dorsal Root Ganglia

DRGN Dorsal Root Ganglion Neurons

ESCs Embryonic Stem Cells

FGF Fibroblast Growth Factor

FGFRs Fibroblast Growth Factor Receptors

GSK3 Glycogen Synthase Kinase 3

hESCs Human embryonic stem cells

hiPSCs Human induced pluripotent stem cells

hPSCs Human pluripotent stem cells

hBMSCs Human Bone Marrow Stromal Cells

hNPs Human Neuronal Progenitors

KLF4 Krüppel-like Factor 4

NC Neural Crest

NGF Nerve Growth Factor

NPCs Neural Progenitor Cells

PNS Peripheral Nervous System

TGF Transforming Growth Factor

Declarations

Conflict of Interest

No conflict.

Author Contributions statement

Kuangpin Liu and Wei Ma wrote the articles, Jinwei Yang collected the research, Si-Jia Zhang and Wei Liu did the statistics, Kewei Zhu, Jie Liu, Hongjie Wu drew the figures, and Liyan Li provided the funding.

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Data Availability

All data in this study are derived from GSE75582, public databases and websites were used for data analysis, including http://www.webgestalt.org/ https://cn.string-db.org, https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE75582

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Tables

Table 1 The top 10 metabolic pathways of gene enrichment in KEGG analysis

Gene Set	Description	P Value	FDR
hsa04360	Axon guidance	0.0000000018008	0.00000058706
hsa04550	Signaling pathways regulating pluripotency of stem cells	0.0000000060949	0.00000099347
hsa05200	Pathways in cancer	0.000000046026	0.0000050015
hsa04512	ECM-receptor interaction	0.00000032751	0.000026692
hsa04510	Focal adhesion	0.0000074337	0.00037700
hsa04514	Cell adhesion molecules (CAMs)	0.0000074425	0.00037700
hsa04151	PI3K-Akt signaling pathway	0.0000080951	0.00037700
hsa04010	MAPK signaling pathway	0.000026661	0.0010865
hsa04350	TGF-beta signaling pathway	0.000059346	0.0021496
hsa04014	Ras signaling pathway	0.000075788	0.0024707

Table 2 Differential genes in stem cell pathway

Gene Symbol	Expression
ACVR1C BMP4 BMPR1A BMPR1B FGF2 FGFR2 FGFR3 FGFR4 FZD1 FZD4 FZD5 FZD6 FZD10 FZD7 HESX1 ID3 ID4 INHBB JAK3 JARID2 KLF4 LIFR MAPK13 NANOG NODAL PIK3CB PIK3CD POU5F1B REST SKIL SMAD3 SOX2 WNT4 ZIC3	Up
ACVR2A APC GSK3B ID1 INHBE ISL1 NEUROG1 ONECUT1 PIK3R1 PIK3R3 SMAD1 TBX3 ZFHX3	Down

Table 3 Cytoscape software screened the top 10 hub genes

EPC	Degree	MNC	MCC
BMP4↑	BMP4↑	BMP4↑	BMP4↑
SOX2↑	SOX2↑	SOX2↑	SOX2↑
FGF2↑	FGF2↑	FGF2↑	FGF2↑
NANOG↑	NANOG↑	NANOG↑	NANOG↑
SMAD3↑	SMAD3↑	SMAD3↑	KLF4↑
KLF4↑	GSK3B↓	GSK3B↓	SMAD3↑
GSK3B↓	WNT4↑	FGFR2↑	PIK3R1↓
FGFR2↑	FGFR2↑	WNT4↑	PIK3CB↑
NODAL↑	KLF4↑	KLF4↑	FGFR2↑
ISL1↓	NODAL↑	NODAL↑	GSK3B↓

↑ represents upregulated genes↓represents downregulated genes

Figures

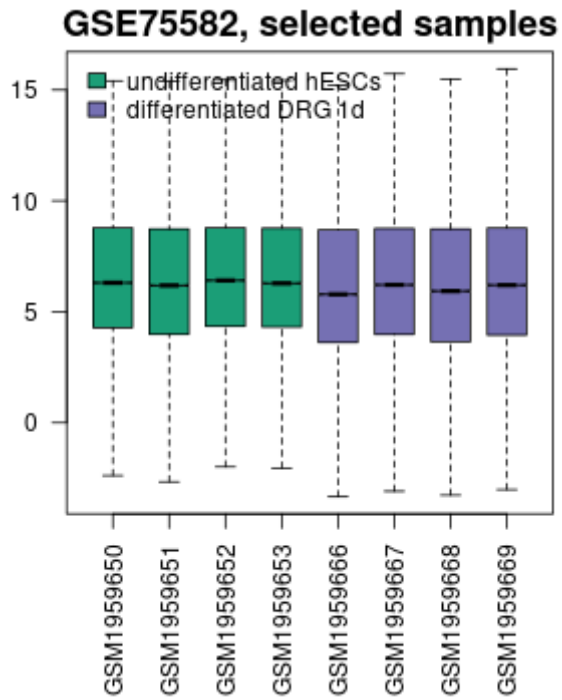

Figure 1

Box diagram (Green: undifferentiated human embryonic stem cells; purple: dorsal root ganglia differentiated by human embryonic stem cells for 1 day)

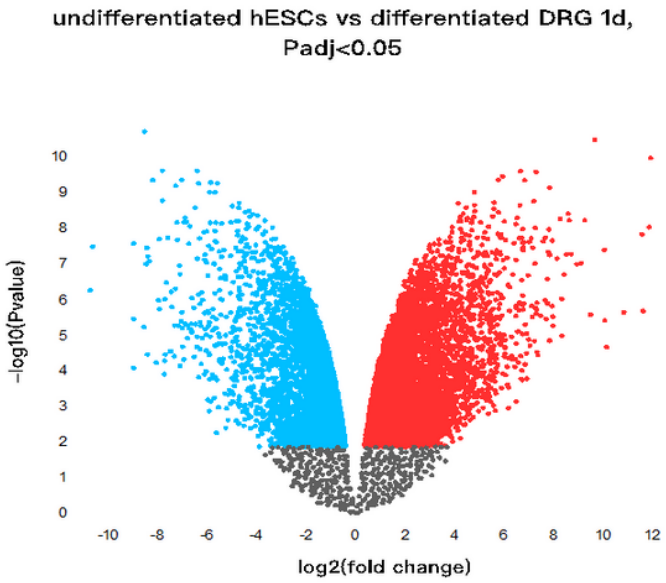

Figure 2

Volcano map (red dot: indicating upregulated genes, blue dots: indicating downregulated genes, black dots: genes with no significant differences)

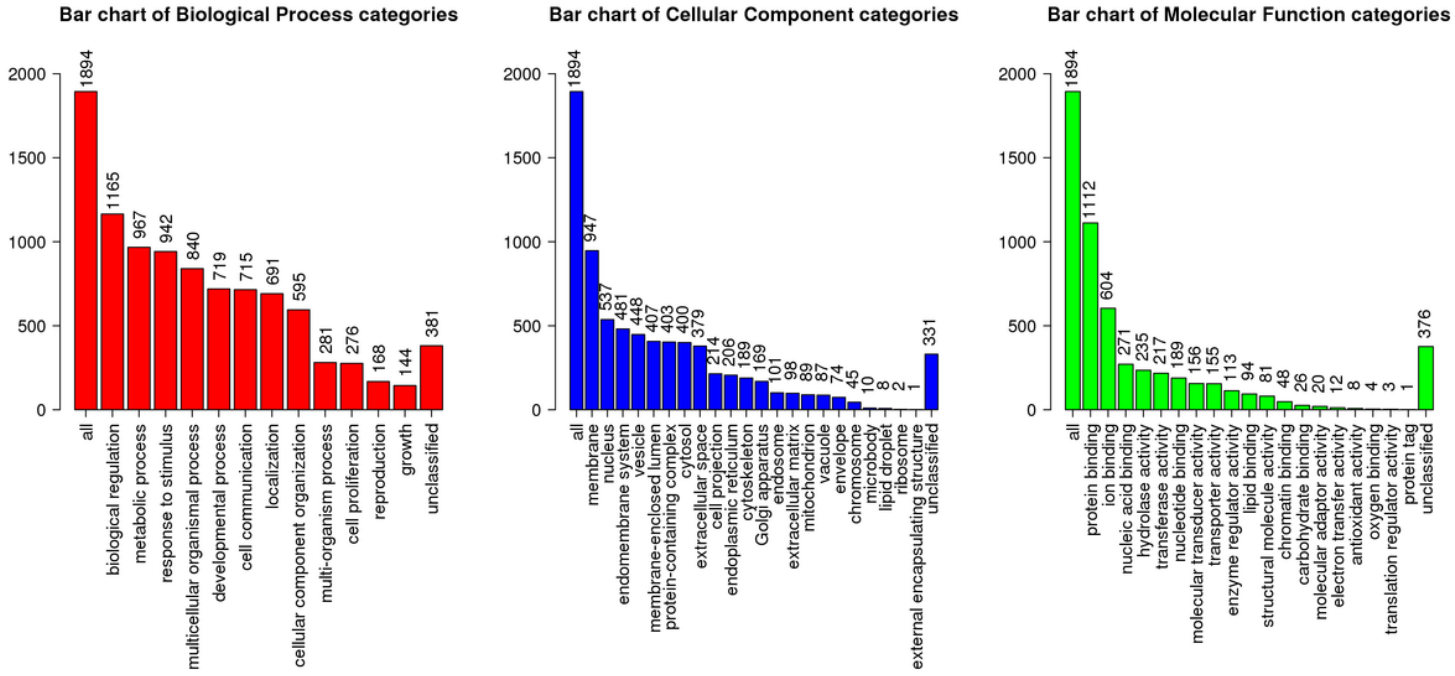

Figure 3

Go analysis of upregulated genes (red: biological processes; blue: cell composition; green: molecular function; ordinate is the number of relevant genes)

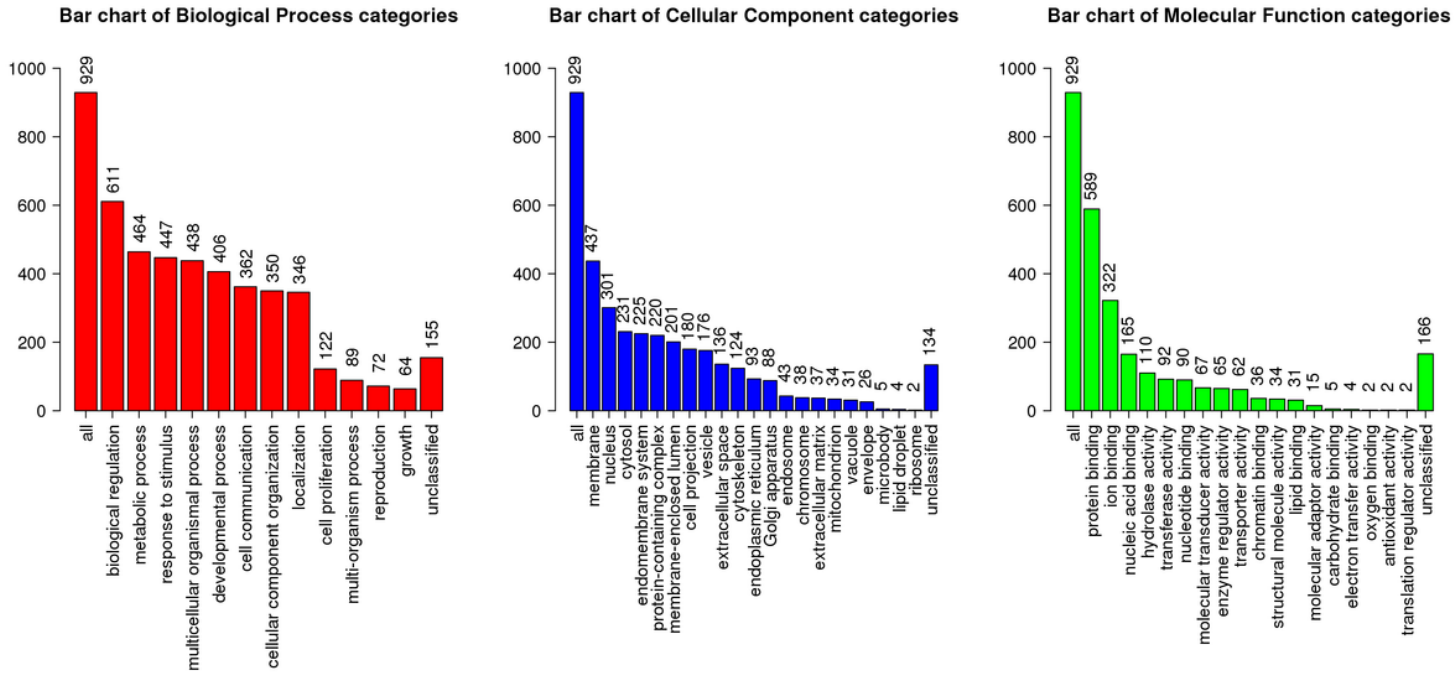

Figure 4

Go analysis of downregulated genes (red: biological processes; blue: cell composition; green: molecular function; ordinate is the number of relevant genes)

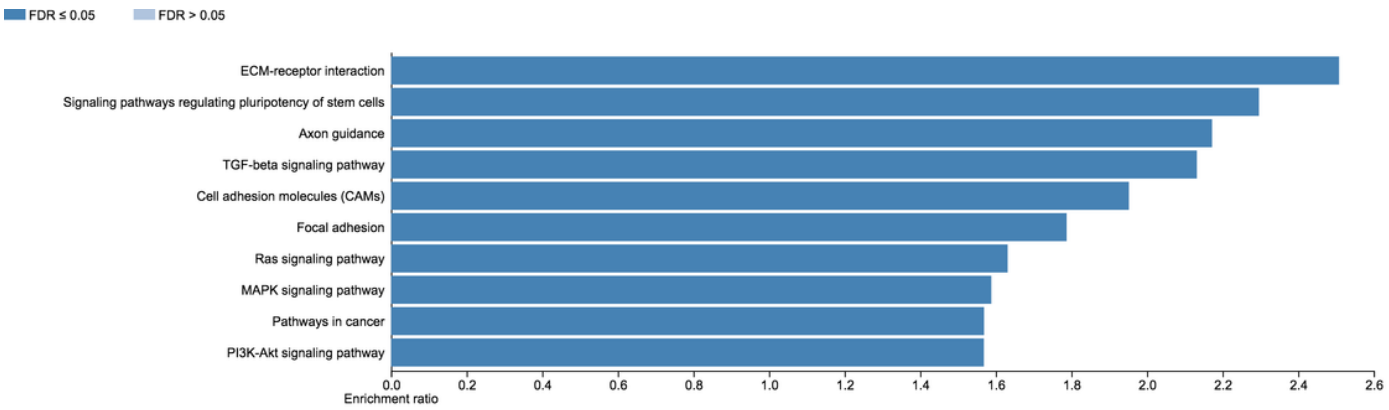

Figure 5

KEGG analyzes the first ten metabolic pathways of gene enrichment

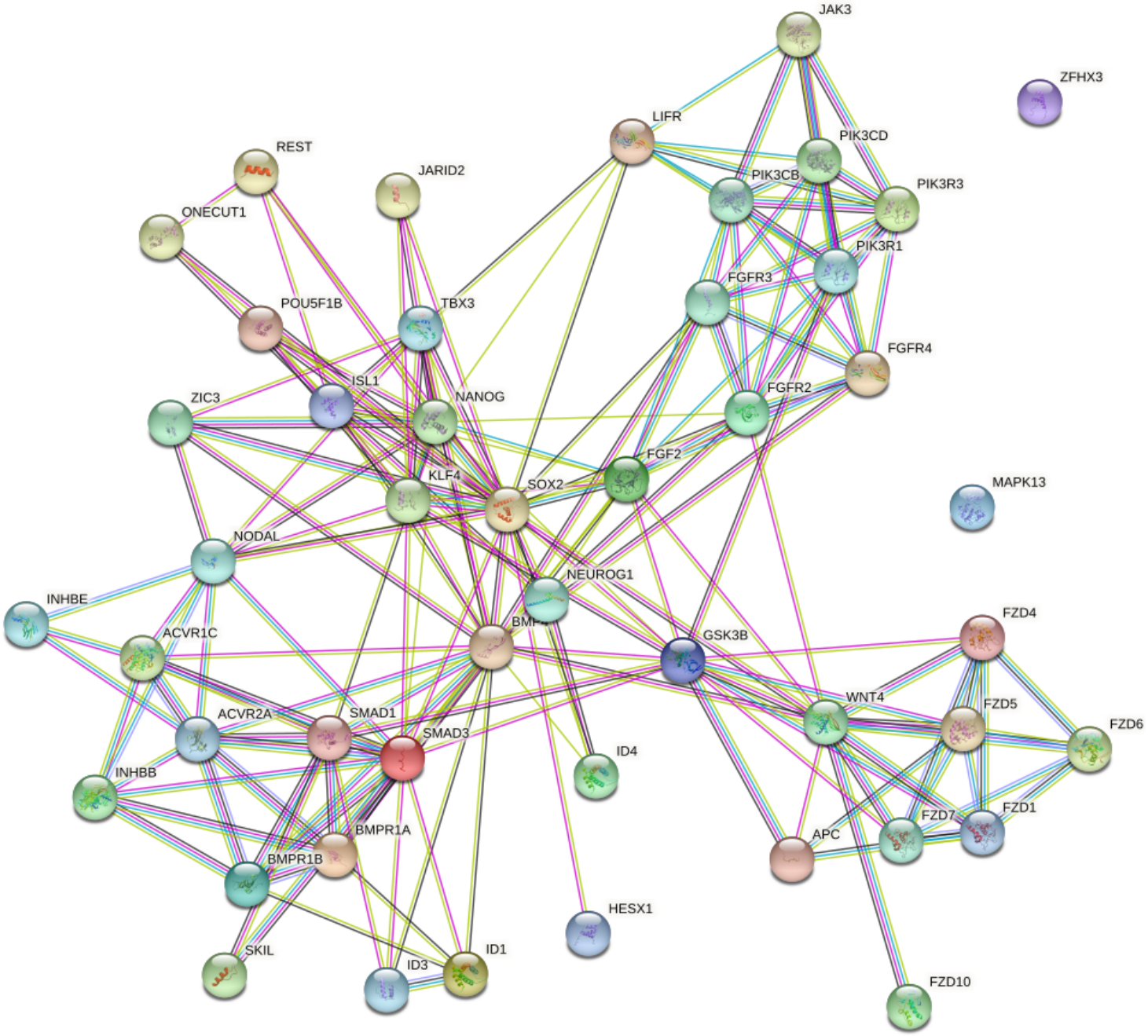

Figure 6

STRING analysis of differentially expressed genes in stem cell-related signaling pathways (different colors represent different genes: —from curated databases—experimentally determined—gene neighborhood—gene fusions—gene co-occurrence; —textmining; —co-expression; —protein homology

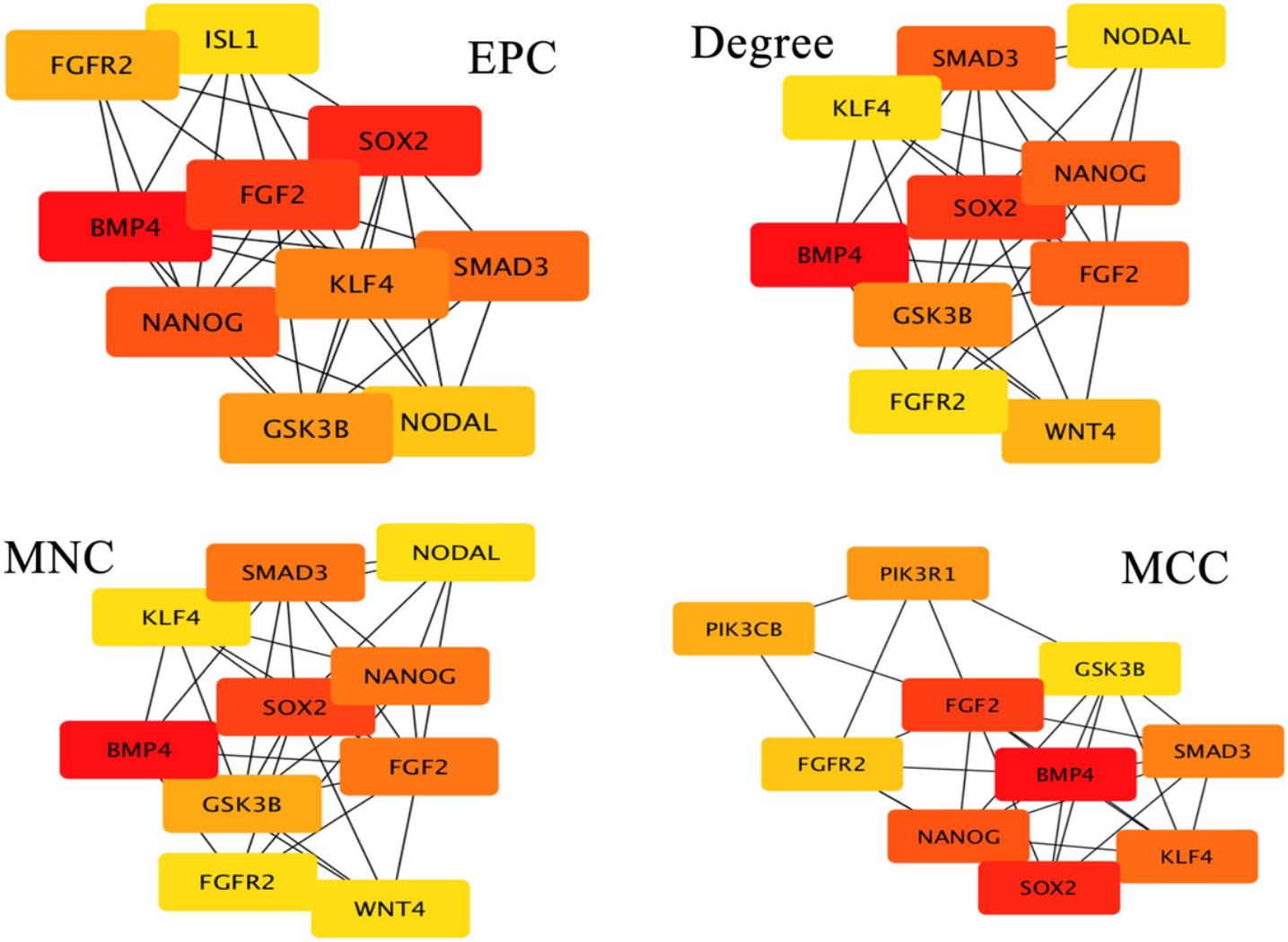

Figure 7

Cytoscape analysis of the top 10 differentially expressed genes in stem cell-related signaling pathways (using algorithms including EPC, Degree, MNC and MCC)