New network analysis of therapeutic targets of human urine stem cells after cerebral ischemia-reperfusion

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Abstract

Objective: In order to verify that human urine stem cells can improve the neurological function of rats with cerebral ischemia-reperfusion through animal experiments, and then analyze the changes of gene network of human urine stem cell transplantation on cerebral ischemia-reperfusion through bioinformatics.

Methods: Human urine stem cells were cultured, and then the model was established in rats with cerebral ischemia-reperfusion. Human urine stem cells were transplanted through the lateral ventricle. The success of the model was verified by behavioral NSS score. Using the keywords "Cerebral reperfusion" and "human urine stem cells", gene targets were searched and downloaded in Genecards, and the downloaded gene targets were made into The Venny intersection plot, and the resulting intersection genes were used. PubMed and CN.ORG searched literatures by keywords "Cerebral ischemia reperfusion, Stem cell transplantation" and sorted out genes that had been reported and not reported in the intersection genes. GO analysis, KEGG pathway analysis and PPI protein interaction map were used to analyze the interaction between genes.

Results: Behavioral NSS score data were obtained on 1 day. Compared with the SHAM group, the neurological function of THE BI rats was significant. After injection of human urine stem cells into the lateral ventricle, the neurological function injury of the BI+USCs=LV group was higher than that of the BI group, P=0.028, which was statistically significant. There were 258 overlapping genes between Cerebral ischemia and human urine stem cells, and the remaining 252 overlapping genes were screened by PubMed and CNKI. GO enrichment analysis mainly involved neutrophil degranulation, neutrophil activation involved in immune response and platelet Positive regulation of degranulation, Hemostasis, blood coagulation, coagulation, etc. KEGG pathway analysis mainly involved Complement and coagulation cascades, ECM-receptor interaction, Hub gene was screened as:CD44, ACTB, FN1,ITGB1, PLG, CASP3, ALB, HSP90AA1, EGF, GAPDH. We predict that these 10 genes have a regulatory role in cerebral ischemia reperfusion

Conclusion: Through animal experiments, it has been verified that human urine stem cells can improve the neural function of cerebral ischemia-reperfusion rats, and by studying their interaction relationship, enrichment analysis and pathway analysis, it has been expounded that human urine stem cells can regulate the functional recovery of cerebral ischemia.

Introduction

Cerebral ischemia is one of the main causes of morbidity and mortality in the world, as well as the most important cause of disease deterioration and patient mortality. Ischemic stroke will lead to neuron damageand the restoration of ischemic areas. This pathological process is called cerebral ischemia reperfusion, and cerebral ischemia reperfusion is a complex multifactorial process(Guo, Jin et al. 2019). Effective reperfusion therapy is limited to approximately 4 hours after cerebral ischemia(Shen, Zheng et al. 2017). Cerebral ischemia can lead to neuronal injury, cognitive function, learning and memory impairment, neurological impairment and brain death. Ischemic stroke is associated with morbidity, mortality and disability. In addition, there is no effective clinical treatment that can improve the prognosis of ischemic stroke at present,(Li, Luo et al. 2021). Thrombolytic therapy as the standard treatment for ischemic stroke. However, reperfusion to restore cerebral blood flow in ischemic brain tissue eventually leads to irreversible brain damage. In addition, a variety of complex molecular mechanisms, including excessive oxidative stress and inflammatory response, calcium overload and apoptotic cell death, are involved in the progression and pathogenesis of cerebral ischemic injury.

Cell therapy is an effective strategy for the treatment of severe neurological diseases, especially ischemic stroke. BMSC transplantation is also an effective strategy for the clinical use of ischemic stroke, and its benefits have been reported(Doeppner and Hermann 2010). BMSC can be used to repair or inhibit neurodegradation by inhibiting cell death and secretion of a series of growth factors as well as anti-inflammatory cytokines in ischemic brain tissue(Huang, Xiao et al. 2021). A number of clinical studies have shown that NSC transplantation is an effective method to treat ischemic stroke through various mechanisms, such as protecting the blood-brain barrier (BBB), alleviating neuroinflammation in the brain, enhancing neurogenesis and angiogenesis, and achieving functional neurological recovery. Experimental studies of NSC transplantation have reported varying therapeutic outcomes, mostly depending on the stage of disease and route of administration(Boese, Le et al. 2018). Although MSC and NSC have been studied and used in some experimental studies and preclinical trials, harvesting MSC and NSC requires invasive medical procedures that may present potential complications, serious ones that may endanger the life of the donor. The ideal source of stem cells would be less harmful to the donor and easier to collect and expand in large numbers.

In view of this requirement, in 2008, Zhang et al. For the first time, stem cells can be isolated from fresh urine samples and will be called human derived urine stem cells (USCs)(Zhou, Benda et al. 2012) . Human-derived urine stem cells can be collected easily without health risks throughout the patient's life cycle, they can even be collected daily(Choi, Chun et al. 2017). These cells can effectively induce ectoderm and mesoderm and endoderm lineages. The ectodermal neural lineage was obtained by adding basic fibroblast growth factor (BGF) to neural induction medium, and about 40% of the induced cells expressed some neural markers and showed neurogenic extension and processes in vitro and in vivo(Ji, Wang et al. 2017). Even though a few cells have a "rice-like" shape in urine, they can continuously multiply to more than 20 generations with a multiplication rate of more than 60. HUSCs can be isolated from fresh urine and propagated in a simple, non-invasive and low-cost procedure(Hwang, Cha et al. 2019, Wu, Soland et al. 2021) , and also have polydifferentiation potential and paracrine action(Huang, He et al. 2022). We found that human urine stem cells (HUSCs) can differentiate into neural lineage cells. When transplanted into the rat brain, HUSCs can survive, migrate, and differentiate into neuronal lineages at the lesion site. There are few studies on direct human urine stem cell (HUSCs) transplantation for ischemic stroke.

In this study, we used animal experiments to verify that injection of human urine stem cells into the lateral ventricle can improve the neural function of cerebral ischemia-reperfusion rats by bioinformatics methods (Wang, Liu et al. 2020) , through gene query and a series of GO, KEGG, PPI analysis (Zhang, Huang et al. 2019). To explore the gene network relationship between cerebral ischemia reperfusion and human urine stem cells, and to provide a new mechanism explanation for whether HUSCs can promote neurogenesis and functional recovery in ischemic stroke.

Materials And Methods

Culture and amplification of human urine stem cells

GO to the hospital for a sterile urine sample of approximately 300ml, followed by cell isolation: After centrifugation, the separated urine was centrifuged at 1500rpm for 10min. After centrifugation, the urine was carefully removed, and the supernatant was gently poured out in the biosafety cabinet. The remaining 0.5ml urine (after centrifugation, all cells would be concentrated by centrifugation) was collected again by urine cell cleaning. Urine cell inoculation, regular observation of pollution and clone formation, USC passage, were performed to

amplify the number of urine stem cells we need, and then use cell immunofluorescence assay to identify human urine stem cells.

1.1.2 Cell viability test: During cell passage, the cells were re-suspended in a 15ml centrifuge tube and centrifuged at 1500rpm for 5min. After centrifugation, the supernatant was discarded and 1ml urine stem cell special medium was added for re-suspension. Then pipette gun was used to absorb 80ul urine special medium +10ul0.4% Trypan blue dye +10ul cell suspension for counting in cell counting apparatus. Then select the counting mode in the cell counting apparatus, click start counting, wait for the instrument prompt "open the top cover of adding liquid", then add liquid, that is, display the result.

Modeling of human urine stem cell therapy for cerebral ischemia-reperfusion in rats

Grouping and administration

Sham group: 7 rats

BI group: 10 rats

BI+BMSC+ lateral brain group: 6 rats

SD rats were provided by Experimental Animal Center of Kunming Medical University, the production license number is: SCXK (Yunnan) K2020-0004and the rats were fasted for 8-10 h

The rats were anesthetized with 3% sodium pentobarbital. The experimental Animal Ethics is KMMU20220891 The common carotid artery, external carotid artery and internal carotid artery on the right side were separated and exposed again by the method of thread embolization. All openings were made in the external carotid artery, and the thread plug was inserted in the common carotid artery. The external carotid artery was dissociated, and then the thread plug was inserted into the internal carotid artery. When the insertion depth was 18 mm, the thin wire at the distal end of CCA was tightly fastened. Finally, close the wound. Observation of single cage feeding. After an hour, pull the tether out. After the completion of modeling, a craniotomy was performed in the right lateral ventricle of the rats, and 1.5mm was opened beside the sagittal line and 2mm backward. Stem cells were injected with stereolocator and microinjector, The number of cells was $1x10^5$ 10ul for each rat, and sealed with bone wax after the injection(Zhang, Liu et al. 2021).

Modified Neurological severity Scale (mNSS)

The three groups of rats were evaluated for neurological severity on 1d,3d,5d,7d,10d,14d, and 21d. There are 5 items in total: 1. Rat tail about 1 meter away from the point (0~3 score); 2. Observe motor function (0~3score); 3. Beam experiment (0~6 score); 4. Sensory function (0~2 score); 5. Reflex activity (0~4 score). Three people will score at the same time, and each person will evaluate one, and the final results will be taken as the average score of three people for statistics.

Query cerebral ischemia-reperfusion and human urine stem cell gene targets

Open your browser, enter Genecars on Baidu, and check the official website. The website is: (https://www.Genecards.org) type in "Cerebral reperfusion" and "Human urine stem" on Genecars Cells "to search and download

the gene target in Excel format. In Genecards database, the higher the Relevance score was, the more closely the target was associated with viral pneumonia, which was then screened and preserved.

Gene cross Venny diagram analysis of cerebral deficiency reperfusion and human urine stem cells

Venny intersection diagram was performed for the genes of cerebral ischemia reperfusion and human urine stem cells to obtain the intersection genes. Search Venny2.1 in Baidu, enter the database, the website is: (https://bioinfogp.cnb.csic.es/tools/venny/), enter to search cerebral ischemia reperfusion respectively corresponding to the gene name and anthropogenic urine stem cells are put inList1 and List2, then changed their names to Cerebral and USCs, respectively, and changed their styles to color downloaded images.

Cross-concentration genes were searched on PubMed by key words

Key words "Cerebral ischemia reperfusion, Stem cell transplantation" were input on PubMed and CNQI to search genes. The reported and unreported genes were screened and sorted into Excel tables.

GO and KEGG were used to analyze all crossed genes.

GO analysis is an international standardized functional classification system of genes. It provides dynamically updated control terms and strictly defined concepts to comprehensively describe the characteristics of genes and their products in any organism. GO enrichment analysis provided all of the GO terms, which were significantly enriched in target genes compared to the genomic background, and filtered corresponding biofunctional target genes. In this study, all targets were mapped to the geneontology database (http://www.geneontology). Count the number of genes per semester. Path-based analysis was used to characterize the biological function of the target. Pathway enrichment analysis in the application of KEGG pathways database (http://www.genome.jp/kegg/) in A target discovered the important signal transduction pathways. In this study, both GOGO analysis and KEGG pathway analysis use R language operation. In this study, R software version 3.6.0 (http://www.r-project.org) is used for GOGO and KEGG analysis. The software needs to be installed in the Java environment, and rSQLite, Cluster Profiler, and org are also required. Related. Dose, enrich Plot, GGplot2, colorspace, stringi, pathView, and so on. Then copy and paste the script for GO analysis and KEGG pathway analysis to the R software window for analysis. After enrichment analysis of GO and KEGG, histogram, bubble diagram and signal pathway diagram were plotted using dB, enrichment diagram and GGplot2.

PPI protein interaction map

Applying string Database (https://string-db.org/), we input 252 common genes to be extracted in the search box of "Name List" of string database, and set biological species to "Homo" in the option of "organization". Then click search. After the next interface is loaded, click continue to make protein interaction map, export and download its high definition interaction map (400PPI) and interaction relationship table.Ten Hub genes were screened according to Degree value in Cytoscape

Results

Human urine stem cells were cultured , amplified and identified. According to the results in Figure 1, urine stem cells expressed CD44 and CD90, but did not express CD45, which was consistent with the stem cell characteristics of urine stem cells in the literature. Then the cell viability of human urine stem cells was tested, as

shown in Table 1 and Figure 2. The cell viability was GOod. On day 2, day 4 and day 6, the P3 generation cells were observed after the resuscitation of different urine cells, and open-field photos were taken at 40 and 100 times. The urine stem cells were mainly adherent and most of them were spindle shaped. Open field photos were taken for 4 x and 10x at upper, lower, left, right and center positions to record the growth of cells after resuscitated. Cell shapes included: round, diamond, spindle and polyGOn cell number. Refraction: GOod; Color: clear; 3. Size, as of a grain of rice; Shape :(spherical, spindle, triangular). Description of cell growth status (adherent shape): pinnate expansion; Less spherical adhesion; Aggregate growth; Length of protuberance (half of cell body); Outgrowth: More outgrowth on both sides, but also on one side. It can be seen from Figure 3 that the cell shape is mainly fusiform. In addition, there are round cells, which may be incomplete or in bad condition.

Table 1 Cell viability test results

Cell viability test results	
Total cell concentration	7.18E+05
Living cell concentration	6.96E+04
Dead cell concentration	2.22E+04
Live cell rate	96.91%
Mean cell size	14.19um
The dilution ratio	2
Cell agglomeration rate	2.105%

Behavioral mNSS score

In order to verify the success of modeling, mNSS neurobehavioral evaluation was performed on the sham group, BI group and BI+USCs+LV group on 1, 3, 5, 7, 10, 14 and 21 days after surgery, respectively. SPSS was used for analysis, univariate measurement anOVA was used for statistics and mapping, and the results were as follows: On day 1, compared with the SHAM group, the neurological function of THE BI rats was significant. After injection of human urine stem cells into the lateral ventricle, the neurological function injury of the BI+USCs=LV group was higher than that of the BI group, P=0.028, which was statistically significant. On day 14, compared with the SHAM group, the neurological function of THE BI rats was significant. After injection of human urine stem cells into the lateral ventricle, the neurological function injury of the BI+USCs=LV group was lower than that of the BI group (P=0.028), which was statistically significant. The results of repeated measurement variance analysis were as follows: Repeated measurement ANOVA was used for analysis and sphericity test, and the results were P=0.000, < 0.05. The data did not meet the sphericity hypothesis. Referring to the correction results of Greenhouse-Geisler, the results showed that both time and time*group had $P < 0.05$, suggesting differences in each time indicator variable. And the effect of treatment factors on index variables will change with the time. The comparison between groups and the test of inter-subject effect gives ANOVA of the treatment factor groups. $P < 0.001$indicated that there was a difference in the amount of difference between different treatment groups. As shown in Figure 4 and Table 2:

Table 2: Repeat measure ANOVA table

Repeat measure ANOVA table					
Variables	DF	SS	MF	F	P
Intervene	2	2306.271	1153.136	233.912	0.001
Intergroup error	20	98.596	4.930		
Time	2.943	141.833	48.193	91.143	0.001
Time*Intervene	5.886	32.503	5.522	10.443	0.001
Repeated measurement	58.861	31.123	0.529		

Note: DF is the degree of freedom, SS is the sum of squares, MF is the mean square and P is the significance P-value.

The effect of urine stem cell transplantation on cerebral ischemia-reperfusion was verified by bioassay. A total of 1493 genes were detected in Genecars for cerebral ischemia reperfusion. Some genes are shown in Table 3:

Table 3 Some gene targets of cerebral ischemia reperfusion

Some gene targets of cerebral ischemia reperfusion							
APP	CBS	KCNJ5	CASP1	NTRK2	TSPO	ARID1B	F8
KRIT1	SLC1A2	ADM	F13A1	CTSD	HTRA2	LPL	IGFBP3
CST3	HMOX1	ODC1	ATP1A2	ITPR1	SLC9A1	EDNRA	IL12A
F2	MMP9	CYCS	AIFM1	FGB	GPT	EDNRB	CCL3
IL6	SELE	PDGFB	ANGPT1	HGF	SLC6A4	SMAD4	CCL5
TNF	EPO	NFE2L2	APOB	TLR2	CSF1R	RELA	HSPB6
NOS3	SELP	MAP2	PARP1	FOS	PF4	TGFBR1	AIF1
COL4A1	S100B	NPPA	FAS	HSPA8	PTGS1	ADA	CHAT
ACE	SOD2	TEK	CSF3	MIR146A	PRKCE	MIR155	OCLN
F5	JAK2	TSC2	HSPA1A	PPARG	PRKAA2	ACTB	CTSL
NOS2	MIR21	CASP9	AQP4	GLUL	TOMM40	DRD2	HMGCR
ENG	SERPINE1	GJA1	SLC6A3	CD40LG	MIR34A	TIMP3	PARK7
SOD1	CXCL8	FLT1	SPTAN1	DLG4	PDP1	MIR145	SPP1
MTHFR	THBD	PON1	G6PD	EPRS1	REN	PIK3CG	MCU
ICAM1	CAT	HSPA5	VCAM1	NPY	PTK2B	CDKN2A	ACTG1
PIK3CA	VWF	AKT1	LTA	SLC1A1	HP	PPARA	CACNA1B
MPO	HSPA4	SHH	MPL	MIR210	SELL	NLRP3	ABCB1
GAD1	ALB	SNCA	CR1	LMNA	ADORA3	CDON	MIR126
TP53	MB	GRIN1	ACHE	PECAM1	TNFRSF1A	AGER	AVP
VEGFA	GRIN2B	AGTR1	ADORA1	ATM	IL17A	FN1	PRKN
PTEN	TGFB2	MAPK14	CALR	ANGPT2	SYNGAP1	MYH7	MUC1
IL10	MMP2	IL18	ELANE	TIMP1	MME	CD40	SERPINA1
MEF2C	IGF1	YRDC	TXN	LOX	NGB	CNR1	CKB
CASP3	ENO2	MTOR	JUN	FLNA	HTR2A	TTN	ADCYAP1
PLAT	NPPB	SMARCAL1	CTLA4	SLC8A1	THBS1	STAT3	CPT2
SMARCA4	F3	PLG	CP	ERCC2	TGIF1	TNNI3	COMT
MAPT	IL1RN	ADAMTS13	EGF	ANXA5	GDNF	MIF	ITGA2B
GFAP	ACTA2	SLC2A1	ADRB2	ITGAM	LAMB1	TNNT2	ADRB1
EDN1	SETD2	APOH	IDH1	CTSB	HSPG2	PCNA	OLR1
PSEN1	SERPINA3	PROC	LDLR	PLAU	MT-CO1	MBP	BGN

SERPINC1	PLA2G6	EGR1	APOA1	NES	RPS27A	NFKB1	FMR1
TLR4	KDR	IFNG	HSPB1	SLC12A2	ADIPOQ	XIAP	NOL3
HIF1A	PTGS2	VLDLR	ALOX5	SMAD2	IL1A	IL13	ENTPD1
PRNP	BAX	INS	SLC17A5	EGFR	NCF1	KCNMA1	SIRT1
CTNNB1	KNG1	AGT	PROCR	LEP	CDKN3	ABCA1	SRC
IL1B	SERPINI1	GRIN2A	THPO	NOTCH1	SH2B3	ITGB2	GLO1
COL3A1	CXCL12	ITGB3	PSAP	BAD	GAPDH	PLA2G7	CA2
CRP	GRIK2	HMGB1	FGA	STAT1	VCP	MAPK8IP1	ABCC8
COL4A2	GP1BA	CALCA	CLU	IL2	AOC3	ITGB1	PRKG1
BCL2	PIK3C2A	FGF2	NGF	BCL2L1	SST	CAMK2A	TIE1
BDNF	GSR	IL4	MAPK8	ESR1	GSS	TERT	CFLAR
XDH	CD36	ADORA2A	TIMP2	IKBKG	FGFR2	BMP6	PDE5A
NOS1	CDK5	MAPK1	MBL2	SMPD1	SDHB	DNM1L	MLC1

A total of 1095 genes of human urine stem cells were found in Genecars. Some genes are shown in Table 4:

Table 4 Human urine stem cells partial gene targets

Human urine stem cells partial gene targets							
IL6	MUC4	UBA1	ACO1	NPHP3	EEF1G	SPPL2A	PROM2
KRAS	IGFBP2	PPT1	MGAT5	CDH16	DDAH1	CAPN7	FUCA2
TERT	C3	LAMP1	GNB3	PPT2	NID2	IST1	SECTM1
BRAF	NEU1	IRAK1	SERPINB9	LBP	LTBP2	KRT75	MYL6B
CD44	ANXA2	DMBT1	AQP2	CCT4	ANXA3	TUBB8	PEF1
CXCL12	TXN	TPI1	ACTR2	ACTN3	ATP6V1A	PYGB	STK25
FGFR3	YWHAE	NECTIN2	DKK3	CFD	H4C12	SERPINA6	H2AC16
ITGB1	STXBP2	MYH10	GPI	UMOD	COTL1	ARHGAP1	EHD4
CDH1	PLA2G2A	KL	SDCBP	EPX	STK26	MGAT1	LRRC57
FGF2	MMP7	HNRNPK	APRT	BAIAP2L1	UPK1A	CLEC3B	GATM
IL1B	SERPINA1	MTHFD1	ARF1	C8B	CFB	GNG4	C8A
CASP8	LGALS1	ARSB	FAT4	NPHS2	CA1	RAB21	MAN1C1
EGF	TUBB	H4-16	HNRNPM	SERPINF2	CACNA2D1	FIGNL1	DOP1B
FASLG	DNASE1	RDX	CETP	SLC12A1	NIBAN1	RAB11B	VPS25
MIR21	APOB	COL18A1	FGB	GSTM3	PYGM	TBC1D10A	GMPPA
CASP3	AQP1	CFL1	HMCN1	VPS4B	UGP2	SMS	PSMA8
PPARG	IGF2R	GP1BA	APOH	TUBB6	TXNDC5	PLPP1	H2AC17
ENG	FCGR3B	ARF6	CKB	FSHB	CAND1	ENO3	NUCB1
VCAM1	KLK3	PGK1	ENPEP	ATP5F1B	SERPINB8	ATP6V1B1	RAB5B
PROM1	KNG1	IGHG1	RBP4	SDCBP2	PACSIN3	NAPRT	PGM2
ABCB1	DMD	GAS6	FABP5	APOA2	PSMB2	ANXA7	GPD1L
HBB	CP	HSP90AB1	EFNB1	MACROH2A1	WASF3	CPVL	TNXA
FN1	PTPRJ	ACTG2	CSTB	MYOF	BASP1	COL15A1	PTTG1IP
CD36	CLTC	MB	LAMB2	SERPIND1	SERPING1	ACTR1A	ENTPD2
THY1	RPS27A	TLN1	GLO1	NIBAN2	GPLD1	LAP3	SPR
MMP9	KRT13	C5	HSPA1B	HPN	GLG1	FAM3C	MYO5C
EPCAM	TTR	LRP2	BST1	SFRP4	MYO1D	ATP6V0D1	H2AC6
MUC1	ACP1	CANX	GPX3	HRG	UPK3A	GCA	RNASE2
NF1	HBA2	YWHAZ	PRDX6	SELENOP	APCS	ARF3	ALDH1L2
ALB	RAB27A	LAMA5	MYO1C	ALDH9A1	ADH5	VPS4A	GUCA2B

RAC1	PRKACA	DAG1	AHCY	LILRB4	SUSD2	MXRA8	PPIC
MMP2	GSN	FGA	PIGR	GM2A	BTD	SULT2B1	H2AC13
SOD1	LAMA3	CNP	ALOX15B	GC	H2AZ1	AKR1C4	PLBD2
ANPEP	RXRA	IGFBP7	HPR	CST6	SORD	PROZ	MON2
SPP1	CFH	CD177	CLIC1	C1R	PAICS	QDPR	CTSZ
LHCGR	PODXL	CDH11	UBA52	MDH1	PAM	MLPH	NAAA
B2M	PRDX1	GGT1	AKR1C3	RHOG	H4C2	ACP2	CUTA
EDN1	KRT1	IGHM	ANO6	FBLN5	LTA4H	LUM	GSTT2
NT5E	PLG	COL4A2	PGM1	DSC1	MPI	H2AC4	CNDP2
SMO	ANXA1	CHI3L1	NID1	ARPC3	SERPINI1	DNPH1	XPNPEP1
ACTB	SERPINF1	CST3	FUCA1	PSME2	NPEPPS	AKR7A2	ATRN
CASP9	KRT20	CHL1	PSMA7	TUBB4B	H2AC20	CPNE2	ABHD14B
FCGR3A	SFN	PABPC1	GANAB	ACLY	SPON2	AOC1	BDH2

Venny diagram

The gene crossover between cerebral ischemia-reperfusion and human urine stem cells was analyzed, and 258 key targets of Venny diagram were obtained by deleting duplicates, as shown in Figure 5:

Cross-concentration genes were searched on PubMed by key words

Key words "Cerebral ischemia reperfusion, Stem cell transplantation" were input on PubMed and CNQI to search genes. Genes reported and unreported in crosssets were screened and sorted into Excel tables. There are 258 intersection targets in the Venny diagram, among which 6 genes have been reported and 252 genes have not been reported, as shown in Table 5, 6, and 7:

Table 5 Cerebral ischemia reperfusion - Human urine stem cells

Cerebral ischemia reperfusion - Human urine stem cells						
CST3	ENO2	TXN	PLAU	ABCB1	GOT2	CDC42
F2	ACTA2	CP	SMPD1	MUC1	TF	LTF
IL6	SERPINA3	EGF	HP	SERPINA1	C4A	ITGAV
ACE	KNG1	IDH1	MME	CKB	ACTC1	EPX
ENG	SERPINI1	APOA1	THBS1	COMT	SERPINF2	FABP1
SOD1	CXCL12	HSPB1	HSPG2	BGN	LRP2	PRDX1
MPO	GP1BA	PROCR	RPS27A	GLO1	FASLG	GGT1
CASP3	GSR	PSAP	GAPDH	SOD3	CTSG	SERPINA4
EDN1	CD36	FGA	VCP	AQP1	NT5E	CD55
SERPINC1	CASP9	CLU	GSS	PROS1	HSP90AA1	SERPINF1
IL1B	PON1	CTSD	ACTB	PLA2G2A	GDF15	TKT
COL4A2	PLG	FGB	FN1	COL18A1	RETN	NID1
MMP9	APOH	HSPA8	MIF	AKR1B1	LGALS3	AHSG
SOD2	AGT	MIR146A	ITGB1	RAC1	ACE2	PTGDS
MIR21	FGF2	PPARG	TERT	LCN2	NAXE	GNA11
CAT	APOB	TIMP1	FABP3	C3	PNP	CD14
ALB	HSPA1A	FLNA	CTSL	NAGLU	SLC9A3R2	CHI3L1
MB	G6PD	ANXA5	PARK7	CASP8	ACP1	C5
MMP2	VCAM1	ITGAM	SPP1	CFH	KL	ALDH9A1
ELANE	CR1	CTSB	ACTG1	IGFBP2	CD44	AOC1
KRT18	GUSB	AXL	PPIA	CD38	MYH10	LIFR
DNM2	S100A8	MGAM	MASP2	HMCN1	THY1	APOC3
VTN	GPX3	PROM1	LDHA	PSMA7	ENTPD2	S100A9
GSTP1	C1R	LRG1	ALDOA	YWHAZ	UBE2D3	HPX
ENO1	AGRN	TNXB	HSPA1B	SELENOP	ATP5F1A	APRT
CAPN1	NEU1	TPI1	GSTA2	HSP90AB1	DBI	IL6ST
NAMPT	APOD	RNASE3	SI	PRDX6	WWP2	LBP
CA1	FCGR3A	F12	PRKACA	ENO3	ACTA1	COL6A1
CRYAB	EPHX2	PLSCR1	CANT1	SAA4	HRG	NPR3
FCGR3B	H2AX	A2M	GSTT1	DDAH2	ACTN4	GC

CD59	CPB2	DDAH1	IGFBP7	FASN	LAP3	ANXA7
DES	IRAK1	CEACAM1	COL6A3	CFB	GSTM3	AMBP
DPP4	EEF2	GOT1	CLEC3B	UMOD	VPS28	SERPING1
PTPA	TOLLIP	LGALS1	LAMC1	SFN	FSTL1	PRDX2
B2M	CUBN	PRDX5	LAMB2	CEACAM5	CBR1	ITCH
TGM2	ANXA2	SAA1	FABP4	PEPD	SELENBP1	SLC3A2
ANXA1	IQGAP1	VDAC1	THBS4	DEFA1	ART3	

Table 6 Gene list was reported after query

Gene list was reported after query		
IL6	IL1B	MPO
SOD1	MMP9	CAT

Table7 No gene list was reported after query

No gene list was reported after query						
CST3	ENO2	TXN	PLAU	ABCB1	GOT2	CDC42
F2	ACTA2	CP	SMPD1	MUC1	TF	LTF
ACE	SERPINA3	EGF	HP	SERPINA1	C4A	ITGAV
ENG	KNG1	IDH1	MME	CKB	ACTC1	EPX
CASP3	SERPINI1	APOA1	THBS1	COMT	SERPINF2	FABP1
EDN1	CXCL12	HSPB1	HSPG2	BGN	LRP2	PRDX1
SERPINC1	GP1BA	PROCR	RPS27A	GLO1	FASLG	GGT1
COL4A2	GSR	PSAP	GAPDH	SOD3	CTSG	SERPINA4
SOD2	CD36	FGA	VCP	AQP1	NT5E	CD55
MIR21	CASP9	CLU	GSS	PROS1	HSP90AA1	SERPINF1
ALB	PON1	CTSD	ACTB	PLA2G2A	GDF15	TKT
MB	PLG	FGB	FN1	COL18A1	RETN	NID1
MMP2	APOH	HSPA8	MIF	AKR1B1	LGALS3	AHSG
ELANE	AGT	MIR146A	ITGB1	RAC1	ACE2	PTGDS
AOC1	FGF2	PPARG	TERT	LCN2	NAXE	GNA11
S100A9	APOB	TIMP1	FABP3	C3	PNP	CD14
IL6ST	HSPA1A	FLNA	CTSL	NAGLU	SLC9A3R2	CHI3L1
NPR3	G6PD	ANXA5	PARK7	CASP8	ACP1	C5
AMBP	VCAM1	ITGAM	SPP1	CFH	KL	ALDH9A1
CR1	CTSB	ACTG1	IGFBP2	CD44	MYH10	TGM2
KRT18	GUSB	AXL	PPIA	CD38	THY1	ANXA1
DNM2	S100A8	MGAM	MASP2	HMCN1	ENTPD2	LIFR
VTN	GPX3	PROM1	LDHA	PSMA7	UBE2D3	APOC3
GSTP1	C1R	LRG1	ALDOA	YWHAZ	ATP5F1A	ANXA2
ENO1	AGRN	TNXB	HSPA1B	SELENOP	DBI	IQGAP1
CAPN1	NEU1	TPI1	GSTA2	HSP90AB1	WWP2	HPX
NAMPT	APOD	RNASE3	SI	PRDX6	ACTA1	APRT
CA1	FCGR3A	F12	PRKACA	ENO3	HRG	SAA1
CRYAB	EPHX2	PLSCR1	CANT1	SAA4	ACTN4	VDAC1
FCGR3B	H2AX	A2M	GSTT1	DDAH2	LAP3	LBP

CD59	CPB2	DDAH1	IGFBP7	FASN	GSTM3	COL6A1
DES	IRAK1	CEACAM1	COL6A3	CFB	VPS28	FABP4
DPP4	EEF2	GOT1	CLEC3B	UMOD	FSTL1	THBS4
PTPA	TOLLIP	LGALS1	LAMC1	SFN	CBR1	GC
B2M	CUBN	PRDX5	LAMB2	CEACAM5	SELENBP1	ANXA7
SLC3A2	PRDX2	SERPING1	ITCH	DEFA1	ART3	PEPD

The GO analysis

To elucidate the mechanism of human urine stem cells on cerebral ischemia-reperfusion, we analyzed GOGO enrichment of human urine stem cell genes extracted during cerebral ischemia-reperfusion. As shown in the figure, the top 10 biological processes (BPS) involved in GOGO enrichment analysis are as follows: Neutrophil activation involved in immune response, neutrophil degranulation, Hemostasis, blood coagulation, negative regulation of coagulation, regulation of coagulation endopeptidase activity Regulation of Peptidase activity. The top 10 factors involved in cell component (CC) in GOGO enrichment analysis were: Vesicle Lumen, Cytoplasmic Vesicle Lumen, Secretory Granule Lumen, Collagen –containing extracellular matrix, blood Microparticle, vacuolar Lumen, platelet alpha granule lumen, platelet alpha granule, endoplasmic reticulum Lumen, Ficolin –1– Rich Granule. The top 10 factors involved in molecular function (MF) in GOGO enrichment analysis were enzyme inhibitor activity, Endopeptidase inhibitor activity and Peptidase regulator Activity, Peptidase inhibitor activity Endopeptidase regulator activity, Sulfur compound binding, Glycosaminoglycan binding, Antioxidant activity, Heparin Binding, Extracellular matrix structural constituent and Protease binding are shown in Figure 6. Figure 6 shows that BP participates in many biological processes, among which neutrophil degranulation is the most abundant biological process. Neutrophil activation involved in immune response, and platelet degranulation. These biological processes constitute an important part of cell development.CC involves many cellular components, among which Vesicle Lumen is the most important chemical component, followed by Cytoplasmic Vesicle Lumen and Secretory granule Lumen, which constitute cellular components related to cell development. MF involves many molecular characteristics, among which enzyme inhibitor activity is one of the most important, followed by endopeptidase inhibitor activity, Peptidase regulator activity, these molecules constitute molecular functions related to cell development (Figure 6).

KEGG pathway analysis

To elucidate the mechanism of human urine stem cells on cerebral ischemia-reperfusion, we analyzed the KEGG pathway after gene crossover (Figure 7). The results showed that the top 10 pathways analyzed by KEGG pathway were: Complement and coagulation cascades, ECM-receptor interaction, Glutathione metabolism, Proteoglycans in Cancer, Legionellosis,

Lipid and atherosclerosis, Staphylococcus aureus infection, Amoebiasis,

Fluid shear stress and atherosclerosis.

PPI protein interaction and analysis

252 intersecting genes not reported in Table 5 were selected as String (https://string-db.org/)After UniProt Transformation), and protein interaction network analysis was performed to understand intermolecular interactions and protein interaction network diagrams. See Figure 8. Each node represents a protein target, and each connector represents the interaction between two target proteins. The larger the node, the higher the centrality of the protein target, the thicker the connecting line, and the stronger the interaction between the two proteins.

As shown in Figure 8, it can be seen that there are many histones and there are complex relationships among genes. Mainly in a circular concentrated in the middle of the protein. Secondly, there is a protein relationship formed at the upper right. There is also a small amount of protein in the upper and lower left.

Screening Hub gene

PPI protein was analyzed by 252 unreported intersection genes, and protein interaction table was derived. Ten Hub genes were screened out according to Degree value in Cytoscape, which were CD44, ACTB, FN1,ITGB1, PLG, CASP3, ALB, HSP90AA1, EGF, GAPDH（Figure 9）

Discussion

We conducted animal experiments to verify that human urine stem cells can improve the neural function of cerebral ischemia-reperfusion rats, and then analyzed the gene network changes of cerebral ischemia-reperfusion induced by human urine stem cells transplantation through bioinformatics. Looking for genes from Genecars, (Safran, Dalah et al. 2010) Venny map was made and genes reported and unreported were searched in PubMed and CNKI. 252 key genes were screened out and used for protein interaction of GO, KEGG and PPI. Then 252 key genes were used to check the Sore values of genes in Gennecars. PPI protein interaction was performed for the genes with top 10 SORes, and the relationship between them was analyzed. According to GO enrichment,(Wang, Zheng et al. 2022) neutrophil degranulation is crucial in BP. In the co-expression module, vesicle Lumen is an important process of cell composition. Neutrophil degranulation is modulated by cell transplantation during cerebral ischemia. In addition, neutrophil degranulation and neutrophil activation involved in immune Response are the terms with the largest number of BP enrichment in our analysis. And enzyme activity is the term with the largest number of MF rich sets. From KEGG pathway analysis, we can see that Complement and coagulation cascades is the most important pathway in KEGG. Hub gene was screened as:CD44, ACTB, FN1,ITGB1, PLG, CASP3, ALB, HSP90AA1, EGF, GAPDH. We predict that these 10 genes have a regulatory role in cerebral ischemia reperfusion

Animal experimental verification

Human urine stem cells can be obtained in a non-invasive way (Bento, Shafigullina et al. 2020). A rat model of cerebral ischemia reperfusion was established, and human urine stem cells were transplanted into the lateral ventricle, and NSS behavioral tests were performed.(Ren, Ma et al. 2021). NSS scores were performed on 1, 3, 5, 7, 10, 14 and 21 days after intracerebral hemorrhage. SPSS statistical analysis and mapping showed that on day 1, the neurological function of BI rats was significant compared with that of sham group. After injection of human urine stem cells into the lateral ventricle, the neurological function injury of BI+USCs=LV rats was higher than that of BI group, P=0.028, which was statistically significant. On day 14, compared with the SHAM group, the neurological function of BI rats was significant. After injection of human urine stem cells into the lateral ventricle,

the neurological function injury of BI+USCs=LV rats was lower than that of BI group, P=0.028, which was statistically significant(Wu, Luo et al. 2020). We hypothesized that human urine stem cells transplanted from the lateral ventricle can survive and differentiate around the infarct, with synaptic protein expression around the cells. Human urine stem cells transplanted at the infarct site can differentiate into functional neurons, which can be substituted by synapse and survival neuron mixing, suggesting that human urine stem cells transplanted into the lateral ventricle for cerebral ischemia-reperfusion rats can promote neural function recovery.(Wu, Luo et al. 2020).

Gene network and its significance

With the rapid development of modern medicine, stem cell transplantation is considered to have great potential in treating neurological diseases, including stroke. Literature has found that mesenchymal stem cells can treat cerebral ischemia and prevent inflammation of glial cells induced by cerebral ischemia by inhibiting TLR4 (Cai, Cai et al. 2021) . Human urine stem cells compared to stem cells from other sources (Pavathuparambil Abdul Manaph, Al-Hawwas et al. 2018). Sources have the following advantages. The collection method from human urine is simple, non-invasive, reproducible and acceptable, and avoids the ethical issues raised by the use of other tissues. Human urine stem cells have been reported to promote stroke recovery (Artegiani, Lyubimova et al. 2017) .However, the molecular network mechanism of urine-stem cell therapy for stroke is unclear. This paper is the first report on the treatment of urine stem cells, and analyzes the network mechanism of stem cell therapy for cerebral ischemia from big data, laying a foundation for the gene network relationship (Fishilevich, Nudel et al. 2017).

PubMed and CNKI screened unreported core genes

So far, the commonly used strategy of bioinformatics is to query targets in the database. Due to the timeliness of genes in the database, previous research summaries have been reported, and the latest PubMed literature results are not included. In this study, we specially supplemented the latest literature results related to urine stem cells. Compared with the results of intersection, 252 intersecting genes that had never been reported in literature were screened out. This study provides important experimental evidence for exploring a new gene network for stem cell therapy of cerebral ischemia. Something added is not reported in the database. This work provides new ideas and methods for perfecting traditional biological information analysis and supplementing new knowledge.

GO enrichment and KEGG pathway analysis

GO enrichment analysis showed that neutrophil degranulation was crucial in BP. Neutrophils can become inflammatory and infected(Mollinedo 2019). According to the literature, after cerebral ischemia-reperfusion, neutrophils will accumulate in the pia meningeal and perivascular space, and eventually reach the infarcted brain parenchyma(Otxoa-de-Amezaga, Gallizioli et al. 2019). In the co-expression module, the lumen is an important process of cell composition. After cerebral ischemia, blood brain barrier is destroyed. Endodermal vesicles are the best spatial and temporal indexes(Haley and Lawrence 2017). In addition, enzyme inhibitor activity was the term with the largest number of MF concentrations in our analysis (Alatan, Chen et al. 2021). Activity of enzyme inhibitors may alleviate neuronal damage caused by ischemic stroke(Min, Lü et al. 2017).

KEGG pathway analysis showed that the treatment of cerebral ischemia-reperfusion by human urine stem cells involved the first ten signaling pathways: Complement and coagulation cascades、ECM-receptor interaction、Glutathione metabolism、Proteoglycans in cancer、Legionellosis、Focal adhesion、Lipid and atherosclerosis、

Staphylococcus aureus infection、Amoebiasis、Fluid shear stress and atherosclerosis (Xie, Zhou et al. 2021). The metabolic network we construct will help select molecular targets and elucidate the molecular mechanisms of cerebral ischemia(Liu, Tang et al. 2016). Complement and coagulation cascade are the most important pathways in KEGG. Adjust is the highest, Count is the highest, and differential expression is the most significant. It is believed to be closely related to the treatment of cerebral ischemia-reperfusion by human urine stem cell transplantation and may be involved in its occurrence and progression(Fang, Zeng et al. 2021).In complement and coagulation cascade signaling, coagulation cascade is used to target proteolysis on the surface of activated platelets. If platelets are activated by exposure to an activated endothelium, they are released to promote the formation of microvesicles that bind to platelet adhesion mediators, clotting factors, and adjacent receptors on the membrane, hydrolysis of the proenzyme cascade proteins into active enzymes, and thrombin production(Green 2006). The occurrence of coagulation cascade may promote cerebral ischemia through thrombin (Xie, Zhou et al. 2021). In the ECM-receptor interaction signaling pathway, ECM-receptor interaction may promote the formation of human urine stem cells. In glutathione metabolic signaling pathways glutathione can be exported and imported through the plasma membrane of many cells (Oestreicher and Morgan 2019). Glutathione metabolism plays a role in shaping the immune microenvironment.(Xiao and Meierhofer 2019) . Among the proteoglycan signaling pathways in cancer, proteoglycan inhibits tumor growth in cancer.(Wei, Hu et al. 2020)、In the Legionnaires' disease signaling pathway, legionnaires' disease may be a pathogen produced after human urine stem cell transplantation (Mikulska, Tridello et al. 2021). An important step in cell migration is adhesion to the substrate with specific adhesion points in the adhesion spot signaling pathway (Paluch, Aspalter et al. 2016). In the lipid and atherosclerotic signaling pathways, atherosclerosis, as a chronic inflammation of the artery wall, is widespread, presenting damage and plaque accumulation in the intima of the artery wall. At the same time, plaque erosion and rupture can lead to blood clots, and atherosclerosis may develop in the brain after their cells are transplanted(Poznyak, Grechko et al. 2020).

Staphylococcus aureus infections in the signal path, staphylococcus aureus, as a kind of symbiotic organism infection, may be accompanied by anthropogenic urine infection occurs in the process, stem cell transplants in amoebiasis signaling pathways, amebiasis was dissolved in the organization amoebic caused in the gut of native animal diseases, the anthropogenic urine amoebiasis disease may occur after stem cell transplantation. Fluid shear stress induces apoptosis of human urine stem cells in both fluid shear stress and atherosclerotic signaling pathways, and atherosclerosis may lead to thrombosis in cerebral ischemia (Rennier and Ji 2013).

Protein interaction and its significance

This paper analyzes the network interaction between cerebral ischemia-reperfusion and various proteins expressed in human urine stem cells(Vella, Marini et al. 2018, Zhou, Xiong et al. 2021). As you can see, there are a lot of histones, mainly in a round cluster of proteins in the middle. Secondly, there is a protein relationship formed at the upper right. There is also a small amount of protein in the upper and lower left. Among them, CPB2, APOH, MIF, CTSG, TIMP1, MMP2, VTN, APOC3, GAPDH and HRG are the most important proteins(He, Gu et al. 2015). Among the 10 most important proteins, there are close connections between each protein. In addition to the strong interaction between the above proteins, DDAH2 and DDAH1 in the lower left corner and IL6ST in the lower right corner have direct interaction with LIFR. These proteins may indicate the stage of cerebral ischemia-reperfusion interaction with human urine stem cells. These molecular network interpretations play an important role in human urine stem cell transplantation during cerebral ischemia reperfusion. Literature has shown that human urine stem cells contribute to functional recovery of cerebral ischemia, and their molecules are regulated

by genes(Pan, Xu et al. 2021). Hub gene was screened as:CD44, ACTB, FN1,ITGB1, PLG, CASP3, ALB, HSP90AA1, EGF, GAPDH. We predict that these 10 genes have a regulatory role in cerebral ischemia reperfusion

Conclusion

Through animal experiments, it has been verified that human urine stem cells can improve the neural function of cerebral ischemia-reperfusion rats, and by studying their interaction relationship, enrichment analysis and pathway analysis, it has been expounded that human urine stem cells can regulate the functional recovery of cerebral ischemia.

Declarations

Ethical approval

All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University. Approved by the Animal Experiment Ethics Review Committee of Kunming Medical University, the approval number is KMMU20220891

The statement

All experimental methods will be reported in accordance with The Arrival Guide.

Consent to participate:

The human urine stem cells were approved by the Medical Ethics Committee of Kunming First People's Hospital, with the ethics number: YLS2021-55

Agree to make a statement:

Do not apply.

Author contribution statement:

WTH and ZZF designed the study. LN and LWQ conducted experiments, while CJL analyzed data and wrote manuscripts. CJL analyzes the data and writes the manuscript. All authors have read and approved the manuscript. The authors point out that all data were generated internally, without the use of paper mills.

Informed Consent Statement:

All subjects who provided urine stem cells signed informed consent.

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Conflict of Interest: There is no Conflict of interest in this study.

Availability of data and materials:

I declare that the data and materials contained in this manuscript have not been published elsewhere and are available.

Thank you

This paper is completed under the careful guidance of Professor Wang Tinghua. Thanks to Professor Wang Tinghua for his guidance and help in my writing. Professor Wang tinghua's rigorous and careful attitude is worth my learning.

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Figures

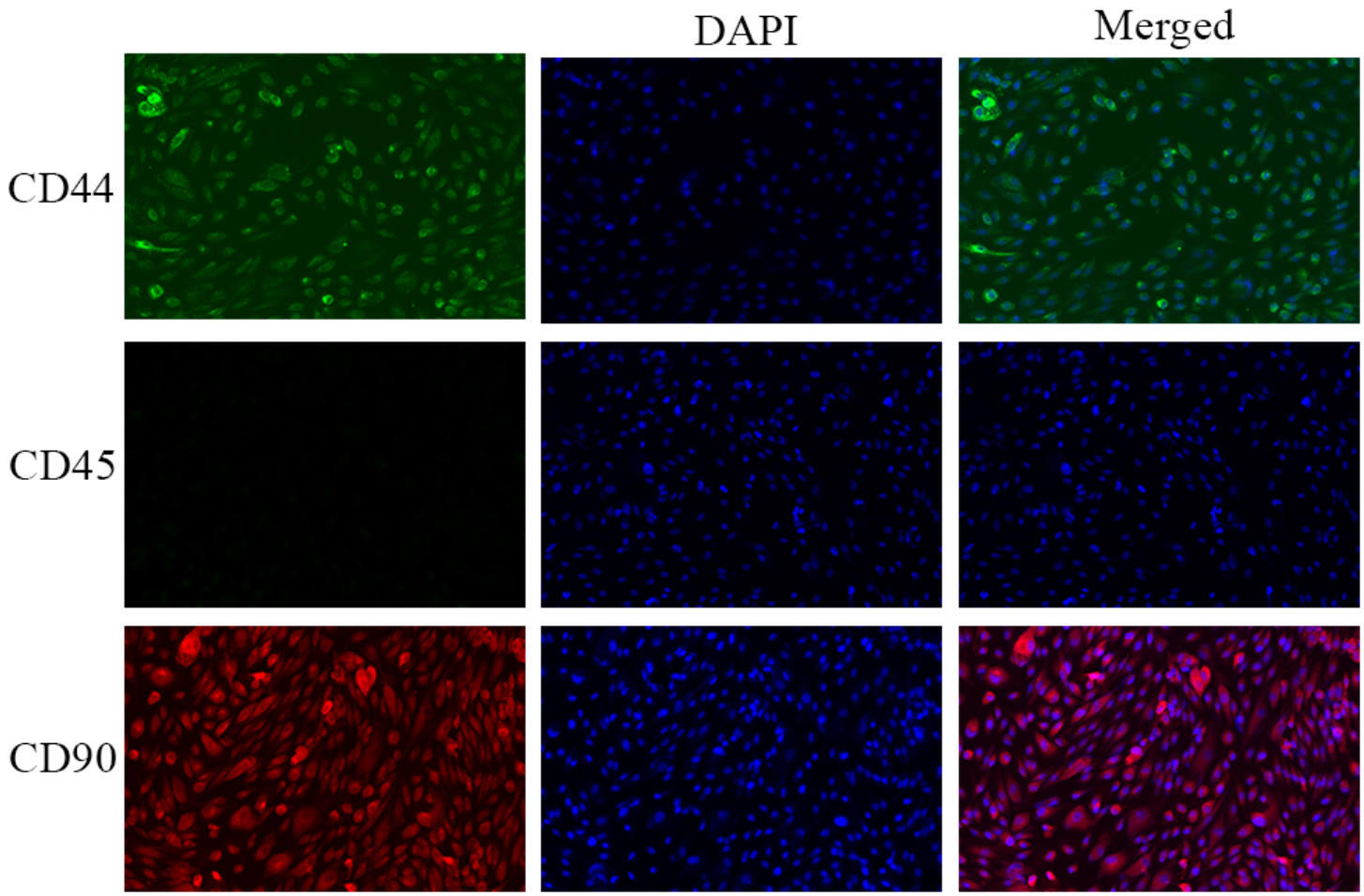

Figure 1

Identification of human urine stem cells

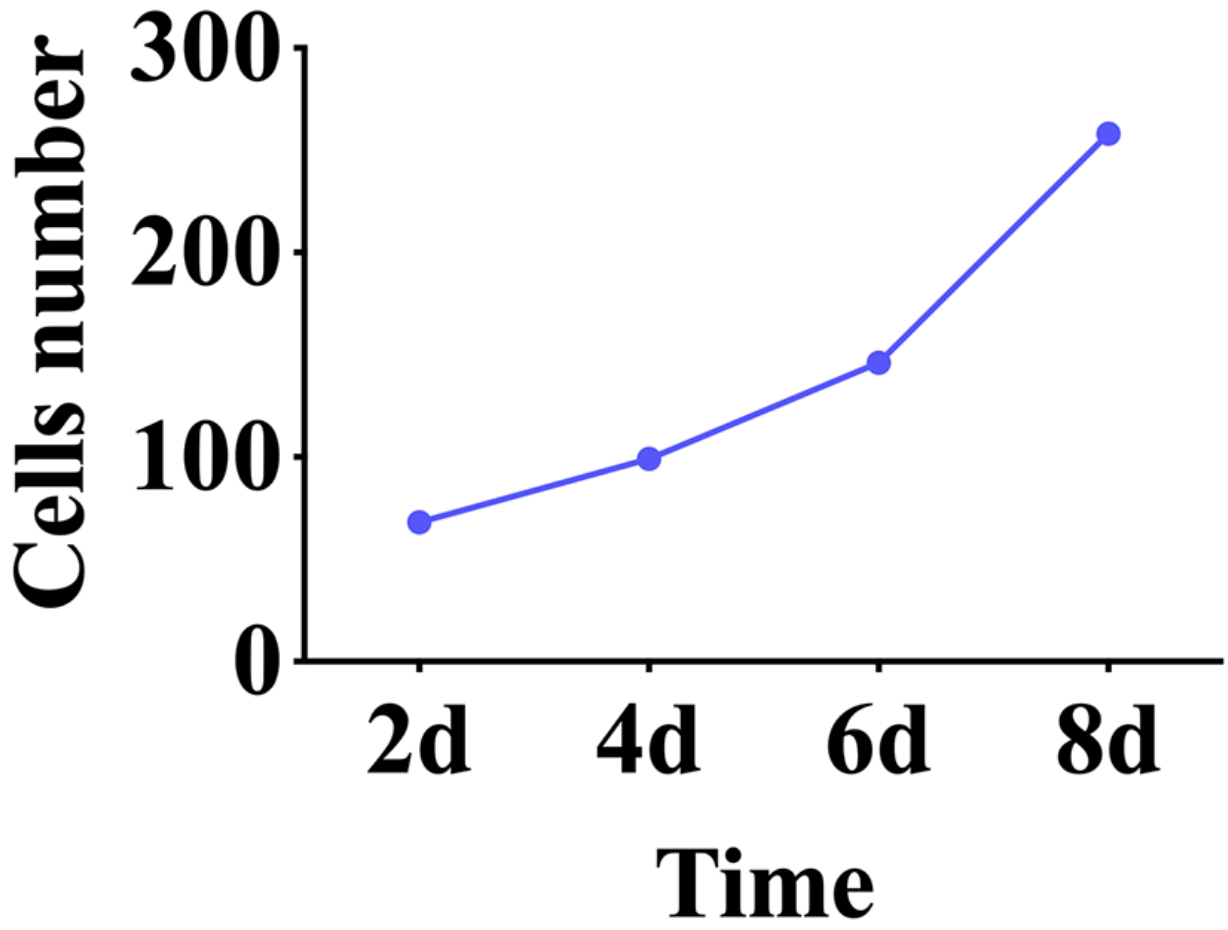

Figure 2

Cell viability test curve

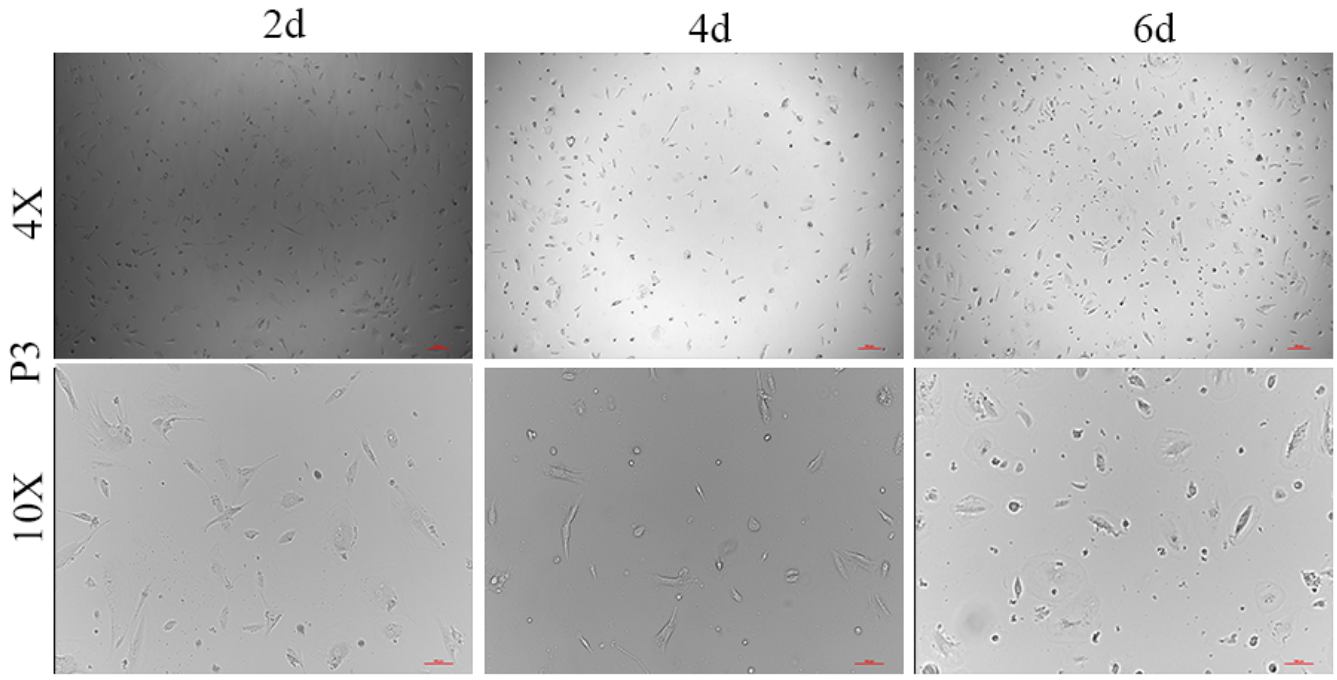

Figure 3

bright field images of different urine cells after resuscitation at 40 and 100 times

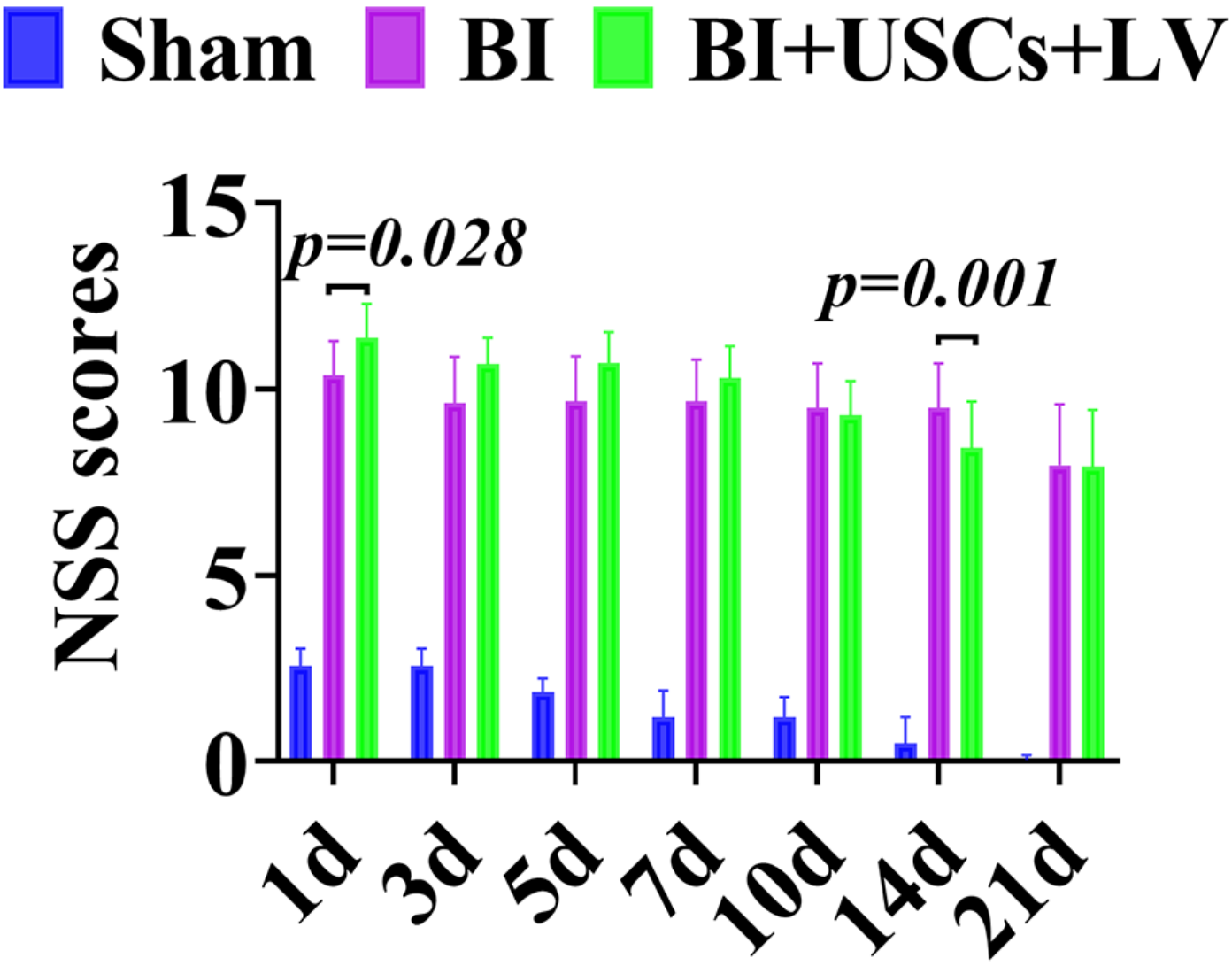

Figure 4

NSS score

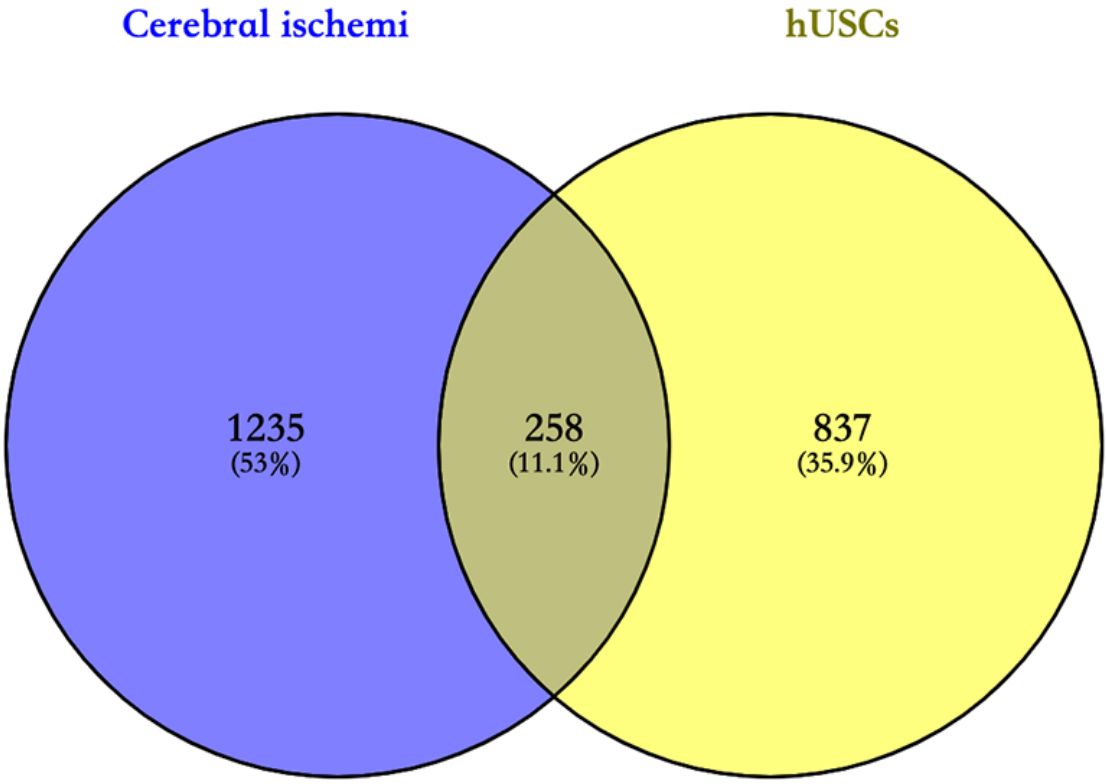

Figure 5

Venny diagram of cerebral ischemia-reperfusion - the key target of human urine stem cells

The left circle is the cerebral ischemia-reperfusion target, the right circle is the human-derived urine stem cell target, in the middle, there is the intersection target between them – the key targets of disease.

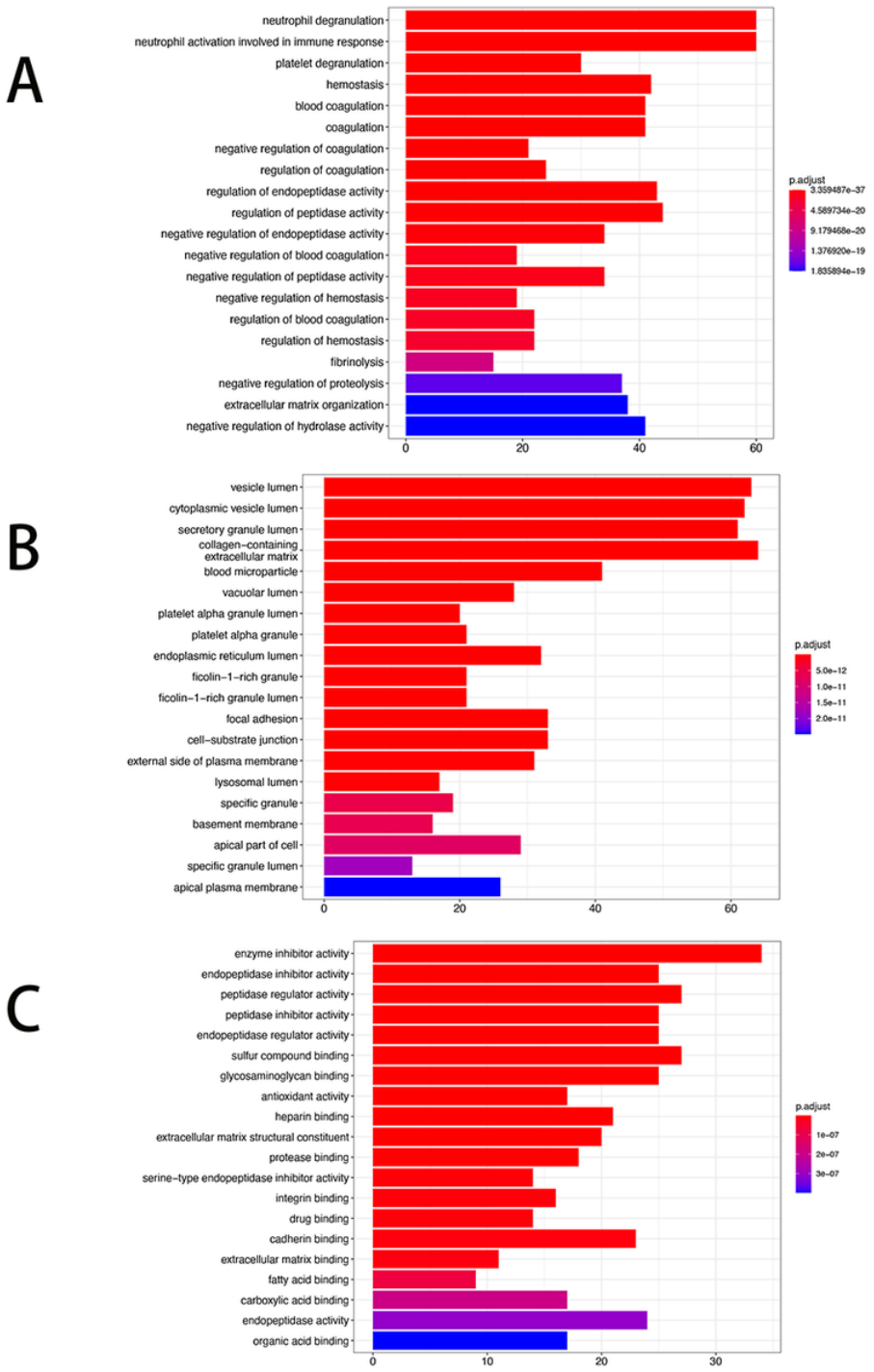

Figure 6

Enrichment diagram of GO analysis. A. Biological processes (BP), B. Cellular components (CC) and C are involved in enrichment analysis. Top 20 factors of molecular function (MF)

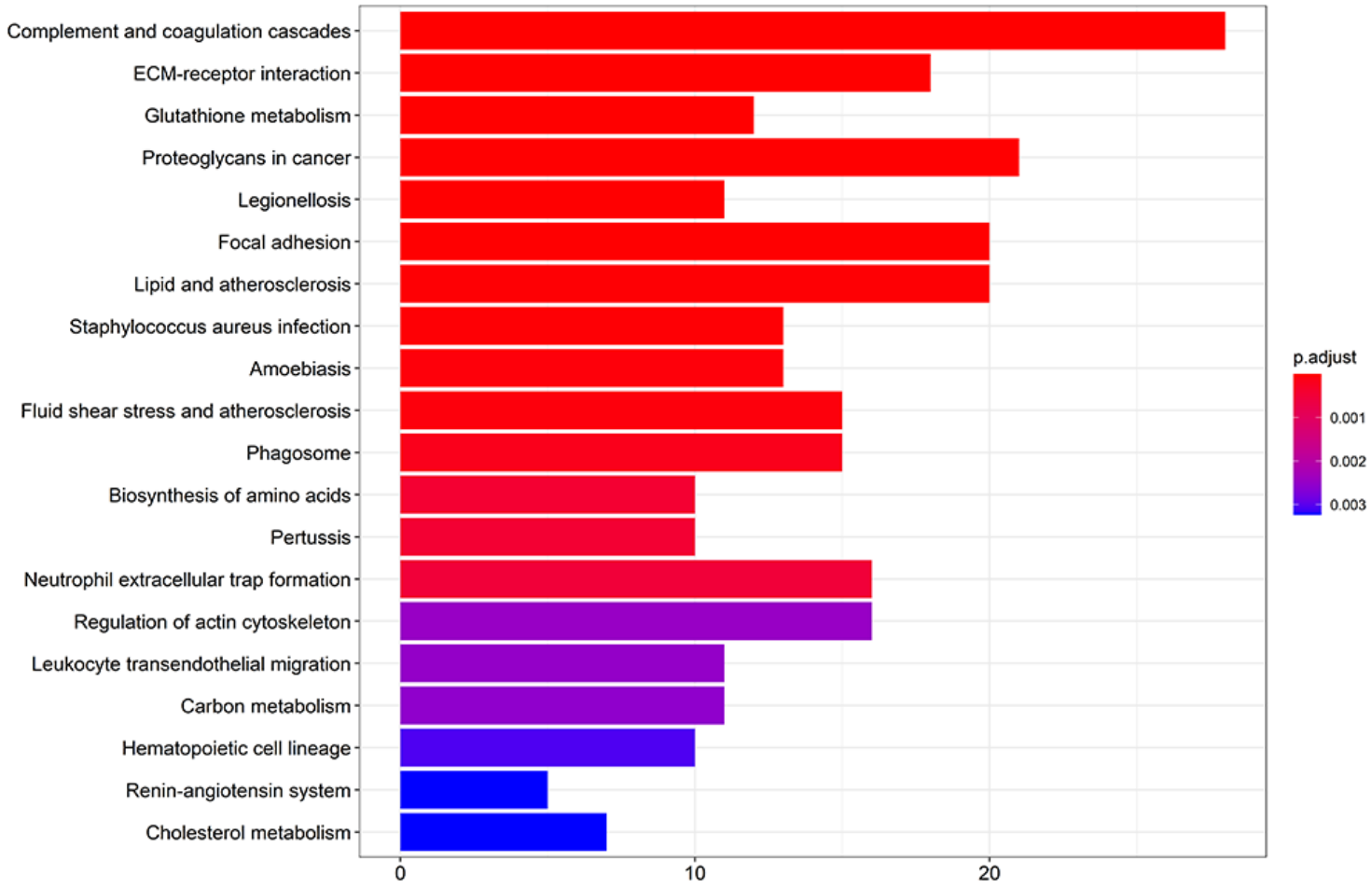

Figure 7

analysis of KEGG pathway

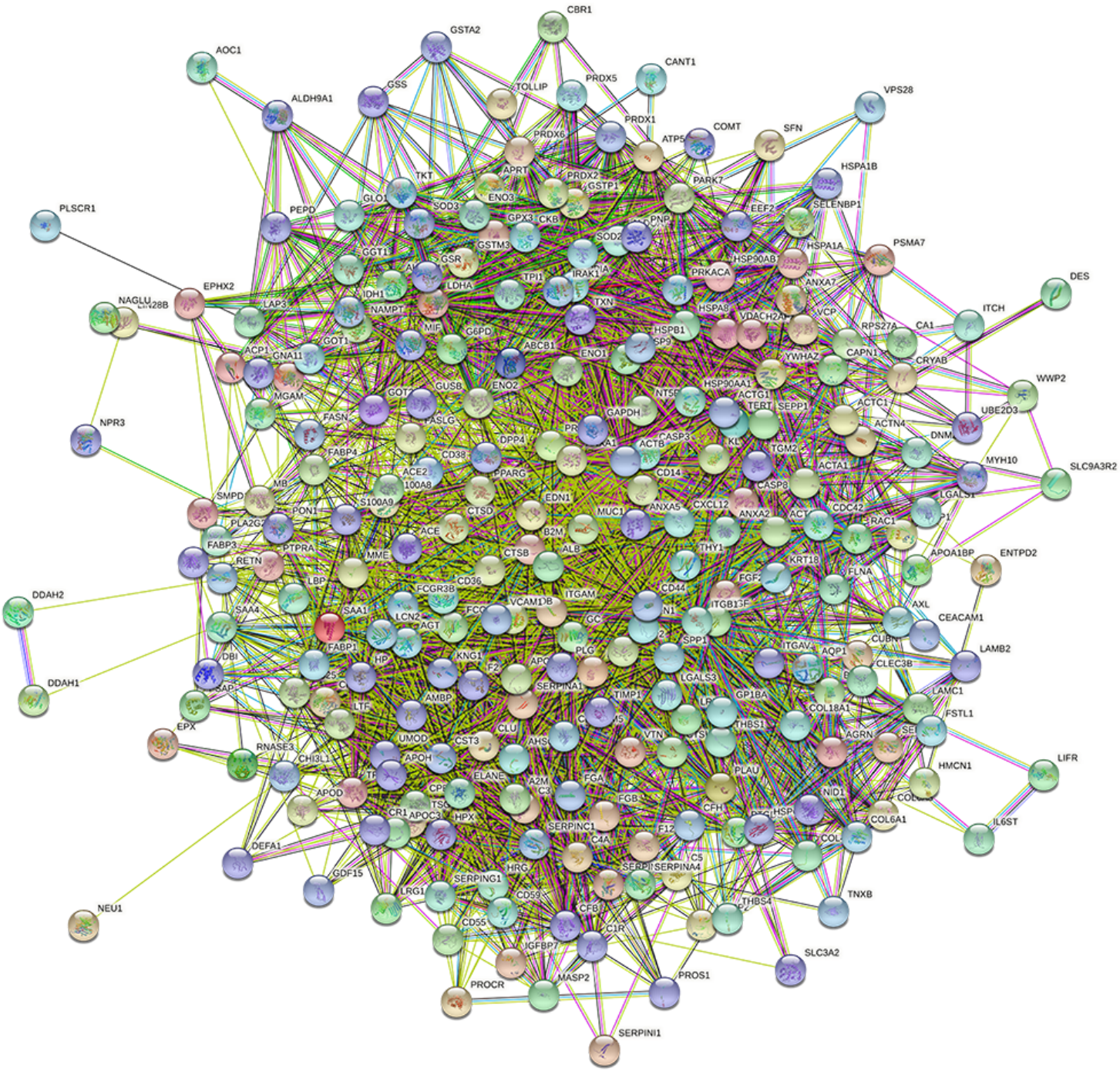

Figure 8

PPI network of genes interaction

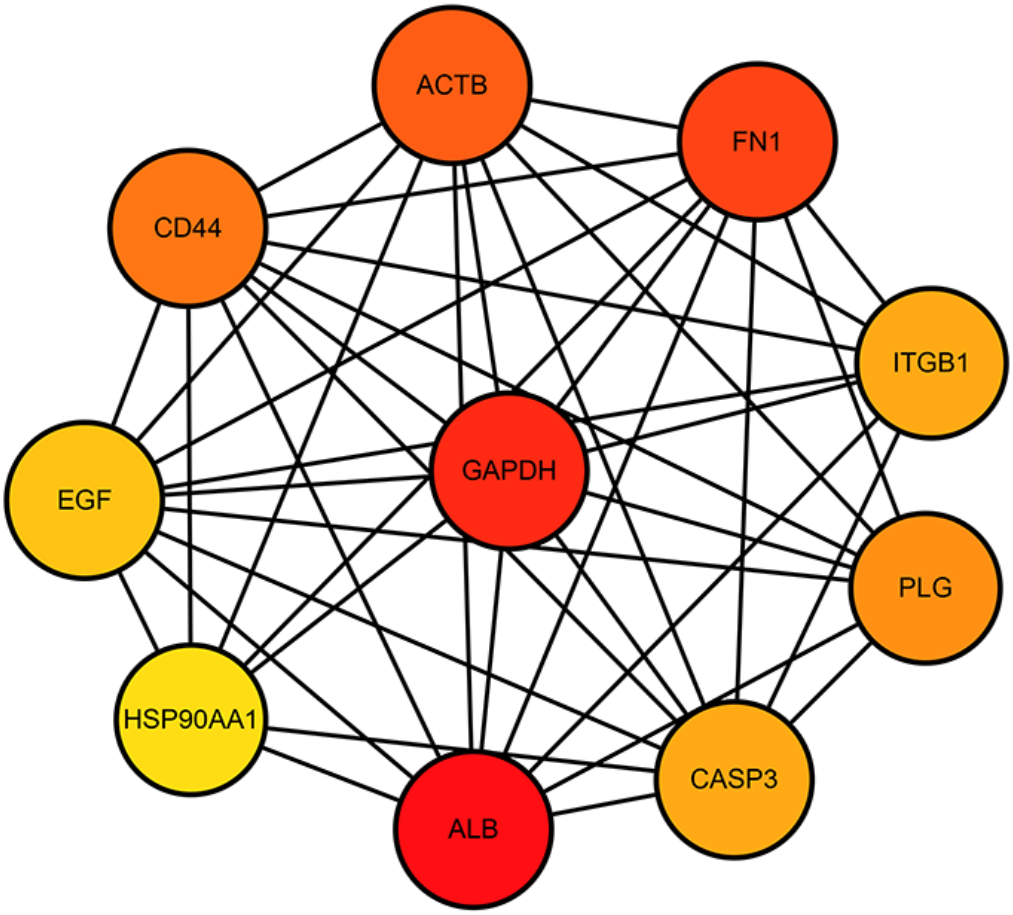

Figure 9

The Hub genes