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Original Article

Bioinformatics investigation of therapeutic mechanisms of Xuesaitong capsule treating ischemic cerebrovascular rat model with comparative transcriptome analysis

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Abstract: Background: Xuesaitong soft capsule (XST) which consists of panax notoginseng saponin (PNS) has been used to treat ischemic cerebrovascular diseases in China. The therapeutic mechanism of XST has not been elucidated yet from prospective of genomics and bioinformatics. Methods: A transcriptome analysis was performed to review series concerning middle cerebral artery occlusion (MCAO) rat model and XST intervention after MCAO from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were compared between blank group and model group, model group and XST group. Functional enrichment and pathway analysis were performed. Protein-Protein interaction network was constructed. The overlapping genes from two DEGs sets were screened out and profound analysis was performed. Results: Two series including 22 samples were obtained. 870 DEGs were identified between blank group and model group, and 1189 DEGs were identified between model group and XST group. GO terms and KEGG pathways of MCAO and XST intervention were significantly enriched. PPI networks were constructed to demonstrate the gene-gene interactions. The overlapping genes from two DEGs sets were highlighted. ANTXR2, FHL3, PRCP, TYROBP, TAF9B, FGFR2, BCL11B, RB1CC1 and MBNL2 were the pivotal genes and possible action sites of XST therapeutic mechanisms. Conclusion: MCAO is a pathological process with multiple.

Keywords: Middle cerebral artery occlusion, Xuesaitong capsule, Traditional Chinese medicine, transcriptome analysis, bioinformatics

Background

Cerebrovascular disease is the second leading cause of death in the globe [1], and also one of the major causes of long-term disability [2]. Current data showed that almost 80% of strokes are ischemic [3], which are resulted from transient or permanent brain artery blood flow reduction [4]. Middle cerebral artery occlusion (MCAO) is the major reason of the blood flow reduction in stroke, and the pathogeny of MCAO is either embolism or local thrombosis.

In the perspective of Traditional Chinese Medicine (TCM), which is independent from Western medicine, embolism and thrombosis are reckoned as blood stagnation. And the treatment of blood stagnation is mainly promoting blood circulation and removing blood stasis, which may be considered as promoting the circulation and dissolving embolus in prospective of Western medicine [5]. Sanqi, also known as radix notoginseng, is an herbal medicine that has been used for vascular diseases for hundreds of years under the guidance of TCM in China. In TCM theory, radix notoginseng could promote the circulation to alleviate the blood stagnation. Panax notoginseng saponin (PNS) is one of the major active ingredients of Radix notoginseng. Researches about PNS have indicated that PNS exerted antioxidant effects, inhibited cell growth, arrested the cell cycle at S phase, and induced apoptosis in cancer cells [6]. Yang et al. had summarized that PNS has significant therapeutic effects and

multiple pharmacological actions including anti-platelet, anticoagulant, antithrombotic, anti-atherosclerosis, lipid-lowering, vasodilative, anti-inflammation, anti-ischemia, anti-arrhythmia, anti-hyperplasia, and promoting angiogenesis effects [7]. To apply the multiple pharmacological effects of PNS conveniently, the Chinese patent medicine, Xuesaitong soft capsule (Kunming Samflaming Pharmacy Group Co., Ltd., Kunming, China) was produced. Xuesaitong soft capsule (XST) is a Chinese herbal monomer preparation consists of PNS, which is mainly used to treat ischemic cardiovascular and cerebrovascular diseases. Modern researches have shown that XST has positive effects on inflammation, adhesion, cell proliferation, apoptosis, and energy supply [8]. Although XST has been proved to have active effects on ischemic cerebrovascular and cardiovascular diseases, there is few analysis and elaboration about XST from perspective of bioinformatics and genomics.

Since the great advances in bioinformatics and genomics, new ways to investigate herbal medicine, TCM formula and Chinese patent medicine are emerging. In this research, we used the technique of bioinformatics and the open access data from Gene Expression Omnibus (GEO), to analysis the genomic pathogenesis of MCAO and the protective effects of XST.

Methods

Identification of eligible gene expression series

Searched microarray profiling series about XST treating rat MCAO model in GEO database, those about rat MCAO model without XST treatment were also searched. Obtained the samples of rat MCAO model and XST treatment after MCAO, those blank rat samples in the same series were also obtained as blank control. The following information was extracted from each identified study: GEO accession number, sample type, platform, number of cases and controls, references, and gene expression data.

Raw data processing

Merged the samples of the same groups and analysis the sample files via R software (Version 3.1.1). Affy package was applied to read the probesets data from the CEL files [9]. Robust Multiarray Averaging (RMA) method was used to normalize the original data. After standardization, a total of 31099 probeset IDs' expression in different samples were obtained.

Screening and annotation of the differentially expressed genes

Used the limma package [10] in R software to compare the probesets expression between blank group and model group, as well as model group and XST group. The threshold was set as $P<0.01$ and fold change>1.5. Annotate package was used to annotate the differentially expressed genes (DEGs).

Enrichment analysis of DEGs

Used Database for Annotation, Visualization, and Integrated Discovery (DAVID) [11] online tools (http://david.abcc.ncifcrf.gov/home.jsp) to annotate and analyze the DEGs. The annotation and analysis were mainly focus on the Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The Threshold was set as $P<0.05$.

Protein-Protein interaction network analysis

Used STRING (Version 9.1) online tools (http://www.string-db.org/) to predict the interactions between the DEGs. Cytoscape (Version 3.1.1) were applied to construct and visualize the Protein-Protein Interaction (PPI) network [12]. Further analysis of the key nodes in the PPI network was processed.

Results

Transcriptome differences between blank rat and MCAO model rat

Two series, Wang L et al (GSE61616) and Glen Jickling et al (GSE21136) were selected and retrieved. The data samples of the same group were pooled together. Eventually there are 6 samples in blank group, 11 samples in model group and 5 in XST treatment group. Rats in model group experienced 2 hour MCAO and then reperfusion. Those in XST group received XST for 7days after 2 hour MCAO and 24 hour reperfusion.

DEGs analysis between blank group and MCAO model group

By comparing the two group samples of blank group and MCAO model group, 870 genes were differentially expressed and annotated. 287

Table 1. Top 10 up- and down- regulated DEGs between normal and model group

Gene symbol	EntrezID	logFC	*P*.Value	Official gene name	Regulation
CHST12	304322	4.5190513	4.4421E-06	Sortilin 1	Up
TSC22D1	498545	10.121914	9.36238E-06	Fucosidase, alpha-L-2, plasma	Up
VAMP7	85491	6.5281481	1.2119E-05	TSC22 domain family, member 1	Up
RGD1562658	498278	5.5242624	1.38276E-05	Synaptotagmin V	Up
PLD2	25097	5.1459871	1.55892E-05	Similar to RIKEN cDNA 1700009P17	Up
PI16	294312	5.8733731	2.13153E-05	Acetylserotonin O-methyltransferase-like	Up
VCAN	114122	5.969893	2.1684E-05	Phospholipase D2	Up
ASMTL	288527	6.1475452	3.19362E-05	Agrin	Up
FUCA2	292485	5.8077055	4.08049E-05	Aminolevulinate, delta-, synthase 1	Up
SORT1	83576	6.5036117	4.32424E-05	Syntaxin binding protein 5 (tomosyn)	Up
BCL11B	314423	-7.166964	1.99003E-05	B-cell CLL/lymphoma 11B (zinc finger protein)	Down
CHD6	311607	-5.472957	7.04857E-05	Chromodomain helicase DNA binding protein 6	Down
CPSF6	299811	-8.171062	6.67188E-05	Cleavage and polyadenylation specific factor 6	Down
CSNK1G3	64823	-7.752591	9.93061E-05	Casein kinase 1, gamma 3	Down
EIF1AX	302697	-8.408783	4.2051E-06	Eukaryotic translation initiation factor 1A, X-linked	Down
FAM35A	364514	-5.525402	3.75845E-05	Family with sequence similarity 35, member A	Down
GPR68	314386	-7.38355	9.4713E-05	G protein-coupled receptor 68	Down
IL13RA2	171060	-3.569281	1.27473E-05	Interleukin 13 receptor, alpha 2	Down
INSIG1	64194	-7.574916	8.67512E-05	Insulin induced gene 1	Down
LARP1B	310348	-5.936671	7.79299E-05	Similar to RIKEN cDNA 1700108L22	Down

Table 2. Top 10 GO enrichment terms and KEGG pathways between normal and model group

Category	Term	Count	*P*.Value
GOTERM_BP_FAT	RNA processing	42	3.00E-07
GOTERM_BP_FAT	mRNA processing	25	1.30E-04
GOTERM_BP_FAT	Immune response-regulating signal transduction	10	1.90E-04
GOTERM_BP_FAT	Immune response-regulating cell surface receptor signaling pathway	9	3.00E-04
GOTERM_BP_FAT	mRNA metabolic process	26	4.50E-04
GOTERM_BP_FAT	Antigen receptor-mediated signaling pathway	8	4.70E-04
GOTERM_BP_FAT	Immune response-activating signal transduction	9	5.50E-04
GOTERM_BP_FAT	Immune response-activating cell surface receptor signaling pathway	8	1.10E-03
GOTERM_BP_FAT	RNA splicing	19	1.10E-03
GOTERM_BP_FAT	Cellular macromolecule catabolic process	41	1.30E-03
KEGG_PATHWAY	Lysosome	13	3.80E-03
KEGG_PATHWAY	Fc gamma R-mediated phagocytosis	11	7.30E-03
KEGG_PATHWAY	RNA degradation	8	1.20E-02
KEGG_PATHWAY	Glycosphingolipid biosynthesis	4	2.20E-02
KEGG_PATHWAY	Other glycan degradation	4	2.70E-02
KEGG_PATHWAY	Thyroid cancer	5	3.10E-02
KEGG_PATHWAY	Adherens junction	8	3.80E-02
KEGG_PATHWAY	Cell cycle	11	4.00E-02
KEGG_PATHWAY	Neurotrophin signaling pathway	11	4.40E-02
KEGG_PATHWAY	Cysteine and methionine metabolism	5	4.70E-02

DEGs (33.00% of all DEGs) were up-regulated in model group and 583 DEGs (67.00% of all DEGs) were down-regulated. The top 10 obviously up- or down- regulated genes (sorted by *P* value) with fold change>1.5 were listed in **Table 1**.

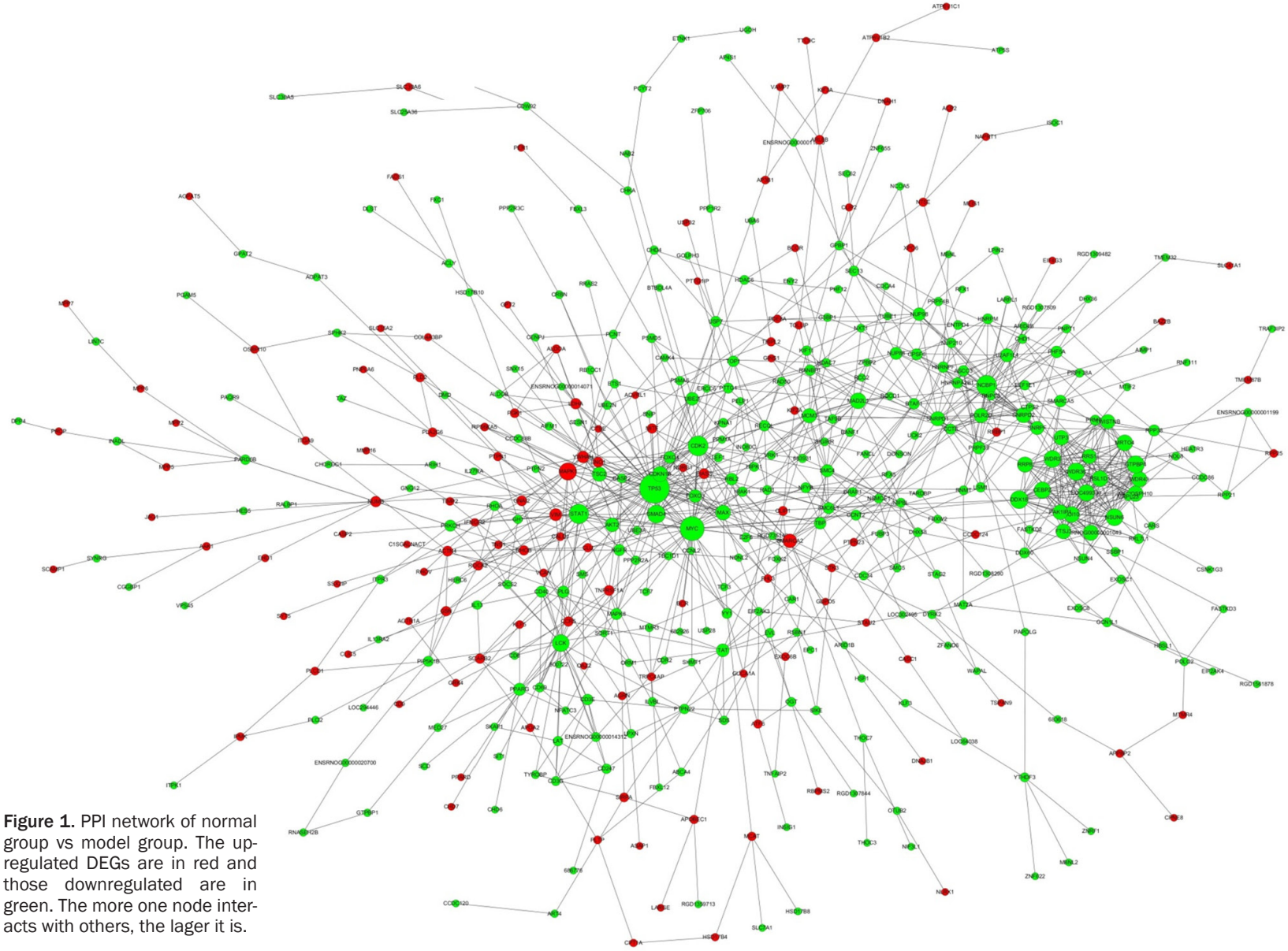

Figure 1. PPI network of normal group vs model group. The up-regulated DEGs are in red and those downregulated are in green. The more one node interacts with others, the lager it is.

Table 3. Top 10 up- and down- regulated DEGs between model group and XST group

Gene symbol	EntrezID	logFC	*P*.Value	Official gene name	Regulation
ARC	54323	7.6650562	2.92612E-05	Activity-regulated cytoskeleton-associated protein	Up
PGK1	24644	11.136091	0.000105978	Phosphoglycerate kinase 1	Up
SFSWAP	304431	5.5658327	0.000179111	Splicing factor, suppressor of white-apricot family	Up
VGF	29461	8.3958614	0.00019166	VGF nerve growth factor inducible	Up
LSS	81681	5.6870309	0.000279211	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	Up
NR4A1	79240	7.2881742	0.000282	Nuclear receptor subfamily 4, group A, member 1	Up
BDNF	24225	6.1445786	0.000288179	Brain-derived neurotrophic factor	Up
ZFP335	259270	5.3598423	0.000292133	Zinc finger protein 335	Up
HOMER1	29546	7.6195098	0.000326317	Homer homolog 1 (Drosophila)	Up
SC5D	114100	8.2294158	0.000369903	Sterol-C5-desaturase	Up
YWHAB	56011	-7.398578	0.000368178	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta	Down
NIT2	288174	-6.044029	0.000499626	Nitrilase family, member 2	Down
COPE	290659	-6.84591	0.0005371	Coatomer protein complex, subunit epsilon	Down
CMTM3	291813	-7.186422	0.000820387	CKLF-like MARVEL transmembrane domain containing 3	Down
PLXNC1	362873	-7.035494	0.000840098	Plexin C1	Down
AASDHPPT	300328	-6.215256	0.001029387	Aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	Down
ANAPC13	685029	-6.264781	0.001040841	Anaphase promoting complex subunit 13	Down
CLN8	306619	-7.006528	0.001115767	Ceroid-lipofuscinosis, neuronal 8	Down
ACAA2	170465	-7.615002	0.00118025	Acetyl-CoA acyltransferase 2	Down
PRCP	293118	-8.080942	0.001242153	Prolylcarboxypeptidase (angiotensinase C)	Down

Functional enrichment and pathway analysis of DEGs between blank group and MCAO model group

To gain insights into the biological functions of the DEGs in MCAO, we performed a GO enrichment and KEGG pathway analysis between blank group and model group. Uploaded the 870 DEGs to the DAVID online tools and set the threshold as P<0.05, 95 GO enrichment terms and 10 KEGG pathways were obtained. The top 10 GO terms and KEGG pathways were listed in **Table 2**.

PPI network construction and visualization

By analyzing the total of 870 DEGs via STRING and cytoscape, 497 genes (nodes) and 1072 gene-gene interactions (edges) were identified. The results were visualized in cytoscape. To modify the PPI network, the sizes of the nodes were set according to their interaction density with other nodes, the color of those up-regulated were in red and those down-regulated were in green (**Figure 1**). The more one gene interacts with others, the larger node it is and the more central this gene occurs within the network. TP53, MYC, CDK2, NCBP1, STAT1, DDX18, SMAD4, GTPBP4, MAPK1, WDR36 are the 10 main genes in the PPI network.

Transcriptome differences between MCAO model rat and XST treatment rat

DEGs analysis between blank group and MCAO model group: By comparing the two group samples of MCAO model and XST treatment after 2h-MCAO and 24 h reperfusion, 1189 genes were differentially expressed and annotated. 1060 DEGs (89.15% of all DEGs) were up-regulated in XST group and 129 DEGs (10.85% of all DEGs) were down-regulated. The top 10 obviously up- or down- regulated genes (sorted by *P* value) with fold change>1.5 were listed in **Table 3**.

Functional enrichment and pathway analysis of DEGs between model group and XST group: GO enrichment and KEGG pathway analysis were performed between model group and XST group. Uploaded the 1189 DEGs to the DAVID online tools and set the threshold as P<0.05, 267 GO enrichment terms and 17 KEGG pathways were obtained. The top 10 GO terms and KEGG pathways were listed in **Table 4**.

PPI network construction and visualization

PPI network was constructed, visualized and modified via STRING and cytoscape as well, 727 genes (nodes) and 1766 gene-gene interactions (edges) were identified (**Figure 2**). SRC,

Table 4. Top 10 GO enrichment terms and KEGG pathways between model group vs XST group

Category	Term	Count	*P*.Value
GOTERM_BP_FAT	Transmission of nerve impulse	69	2.8E-18
GOTERM_BP_FAT	Synaptic transmission	61	5.1E-17
GOTERM_BP_FAT	Metal ion transport	72	3E-13
GOTERM_BP_FAT	Neuron development	59	4E-13
GOTERM_BP_FAT	Neuron differentiation	69	4.4E-13
GOTERM_BP_FAT	Cell-cell signaling	84	6.8E-13
GOTERM_BP_FAT	Cell morphogenesis involved in neuron differentiation	44	8.6E-13
GOTERM_BP_FAT	Neuron projection development	48	5.6E-12
GOTERM_BP_FAT	Neuron projection morphogenesis	43	6.9E-12
GOTERM_BP_FAT	Ion transport	96	1E-11
KEGG_PATHWAY	Amyotrophic lateral sclerosis (ALS)	13	0.00022
KEGG_PATHWAY	Cardiac muscle contraction	15	0.00087
KEGG_PATHWAY	Dilated cardiomyopathy	16	0.0016
KEGG_PATHWAY	Calcium signaling pathway	24	0.0025
KEGG_PATHWAY	Hypertrophic cardiomyopathy (HCM)	14	0.0057
KEGG_PATHWAY	Long-term potentiation	12	0.007
KEGG_PATHWAY	Circadian rhythm	5	0.01
KEGG_PATHWAY	MAPK signaling pathway	30	0.011
KEGG_PATHWAY	Neurotrophin signaling pathway	17	0.012
KEGG_PATHWAY	Phosphatidylinositol signaling system	12	0.013

RAC1, VEGFA, GFAP, GAPDH, CRK, GNB2L1, GRIN1, SCN7A and SST are the 10 main genes in the PPI network.

Profound analysis of transcriptome variation in XST treatment

In the original series, the rats in model group underwent artificial MCAO operation for 2 hours, by comparing the model group and blank group, it is supposed to gain the DEGs related to pathology of MCAO. Those rats in XST treatment group took XST treatment for 7 days after 2 hours' MCAO operation and 24 hours' reperfusion, DEGs between XST treatment group and model group are related to the XST treatment. By merging the two sets of DEGs we obtained above, we might get a profound analysis of transcriptome variation in XST treatment and explore the therapeutic mechanism of XST.

There were 82 genes that differentially expressed significantly in both comparisons. Among those 82 genes, 4 of them were up-regulated in model group and down-regulated after XST treatment, 23 of them were down-regulated in model group and up-regulated after XST treatment, 42 genes were up-regulated in model group and higher after XST treatment, and 13 genes were down-regulated in model group and lower after XST treatment. For the 4 up-to-down DEGs and 23 down-to-up DEGs, they might be the key genes that could elucidate the therapeutic mechanism of XST. Details of these 27 genes were listed in **Table 5**.

Discussion

Comprehensive whole-genome screen of the transcriptome has previously suggested that disease-specific mechanisms are distinguishable and may offer diagnostic and prognostic value [13-15]. In this research, we compared transcriptome profiles from blank group and MCAO model and XST treatment rats to demonstrate the pathology of MCAO and the possible therapeutic mechanism of XST. By the expression profiling between blank group and model group, we might gain access to the pathology of MCAO in genomic level. The comparison between model group and XST group and further analysis of DEGs variation might lead to a profound understanding of XST in treating ischemia cerebrovascular diseases.

The analysis between blank group and model group indicated that MCAO is a multiaspect

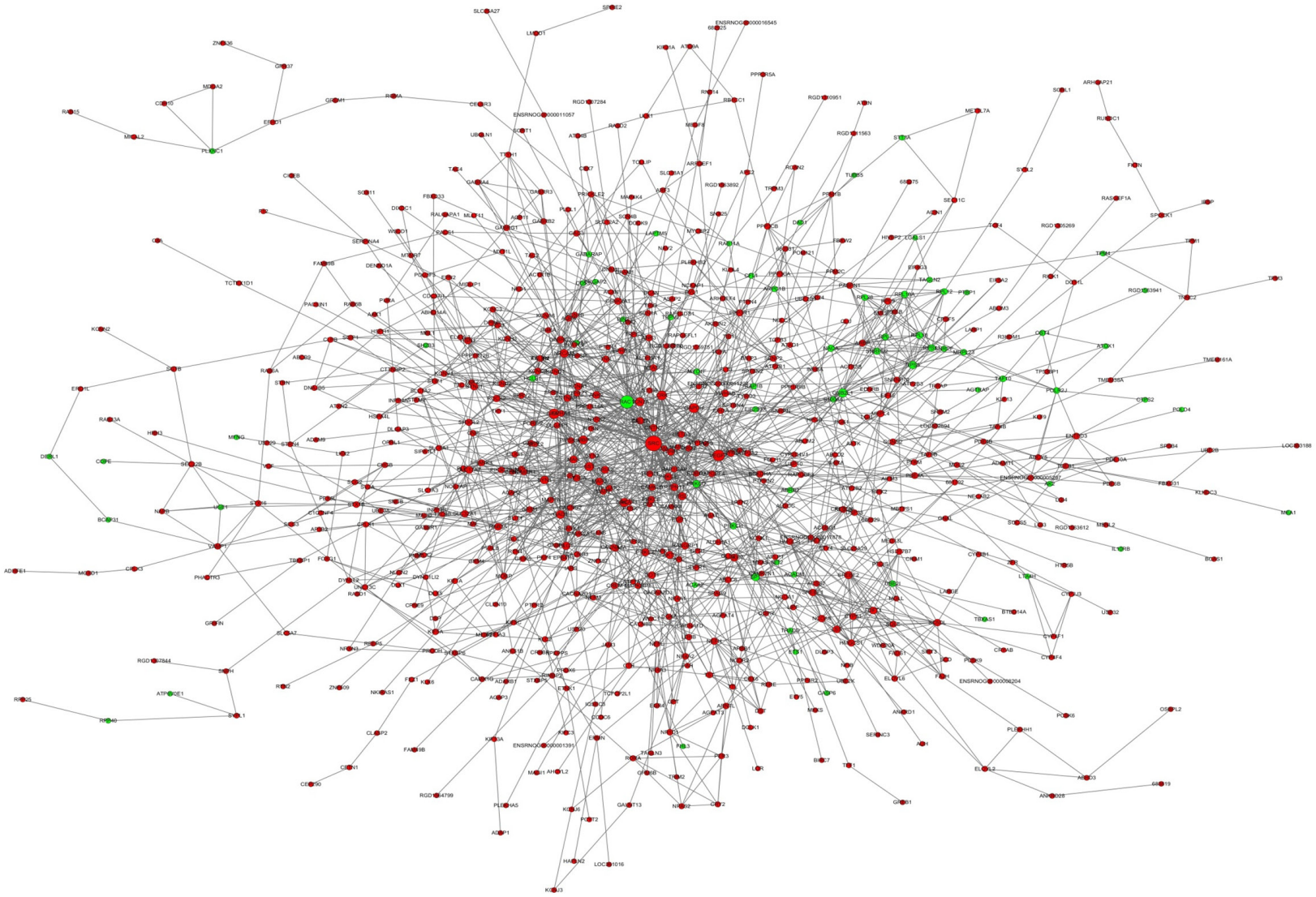

Figure 2. PPI network of model group vs XST group. The upregulated DEGs are in red and those downregulated are in green. The more one node interacts with others, the lager it is.

Table 5. Details of key genes in the combination of DEGs

Gene symble	Official gene name	Variation type
ANTXR2	Anthrax toxin receptor 2	Up-to-down
FHL3	Four and a half LIM domains 3	Up-to-down
PRCP	Prolylcarboxypeptidase (angiotensinase C)	Up-to-down
TYROBP	Tyro protein tyrosine kinase binding protein	Up-to-down
AGPAT3	1-acylglycerol-3-phosphate O-acyltransferase 3	Down-to-up
BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	Down-to-up
CBX7	Chromobox homolog 7	Down-to-up
COX6A1	Cytochrome c oxidase, subunit VIa, polypeptide 1	Down-to-up
ETNK1	Ethanolamine kinase 1	Down-to-up
FBXW2	F-box and WD repeat domain containing 2	Down-to-up
FGFR2	Fibroblast growth factor receptor 2	Down-to-up
HIGD1A	HIG1 hypoxia inducible domain family, member 1A	Down-to-up
ITPK1	Inositol 1,3,4-triphosphate 5/6 kinase	Down-to-up
KCNC3	Potassium voltage gated channel, Shaw-related subfamily, member 3	Down-to-up
LARP1	La ribonucleoprotein domain family, member 1	Down-to-up
MBNL2	Muscleblind-like 2	Down-to-up
MICAL2	Microtubule associated monoxygenase, calponin and LIM domain containing 2	Down-to-up
PAXBP1	PAX3 and PAX7 binding protein 1	Down-to-up
PCYT2	phosphate cytidylyltransferase 2, ethanolamine	Down-to-up
PNISR	PNN-interacting serine/arginine-rich protein	Down-to-up
RB1CC1	RB1-inducible coiled-coil 1	Down-to-up
RSPO3	R-spondin 3 homolog (Xenopus laevis)	Down-to-up
SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	Down-to-up
TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor	Down-to-up
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9	Down-to-up
UBE3A	Ubiquitin protein ligase E3A	Down-to-up
ZFP68	Zinc finger protein 68	Down-to-up

pathological process. Based on DAVID online analysis, many fundamental biological processes were involved. RNA processing (GO: 0006396, P=0.0000003), mRNA processing (GO:0006397, P=0.00013), mRNA metabolic process (GO:0016071, P=0.00045) and RNA splicing (GO:0008380, P=0.0011) were significantly enriched in GO terms. KEGG pathway enrichment shown some specific pathways, including Lysosome (P=0.0038), RNA degradation (P=0.012), Adherens junction (P=0.038) and Neurotrophin signaling pathway (P=0.044). The PPI network showed the gene-gene interactions among identified DEGs, and those key nodes in the PPI network might play important roles in the pathological process of MCAO. TP53 (tumor protein p53) interacted the most with other genes in the PPI network. It is reportedly that TP53-induced glycolysis and apoptosis regulator (TIGAR) plays a neuroprotective role in brain ischemia via enhancing pentose phosphate pathway flux and preserving mitochondria function [16]. CDK2 (cyclin-dependent kinases 2) may be associated with the death of neurons after brain ischemia, reflecting aberrant entry of neurons into the cell cycle [17]. And CDK2 response is associated with brain injury [18, 19]. STAT1 (signal transducer and activator of transcription 1) is one of the most important signaling molecule transducing signals from the cell surface in response to cytokines or growth factors. It might contribute to morphological arterial wall changes in cerebral vasospasm [20]. SMAD4 (SMAD family member 4) is a cause of hereditary hemorrhagic telangiectasia syndrome, and regulates N-cadherin expression in endothelial cells to stabilize the blood brain barrier [21], Jickling GC et al [22] had found that SMAD4 and several of its target genes were differentially expressed in

strokes that developed hemorrhagic transformation. These key genes in PPI network contribute evidence to the finding that MCAO leads to multiple biological and pathological processes, including cell cycle, apoptosis, hemorrhagic transformation, neurotrophin regulation. To apprehend the pivotal biological and pathological process related to cerebral ischemia diseases could make value to the treatment and prognosis.

The analysis between model group and XST group had identified the potential therapeutic mechanism of XST in ischemia cerebrovascular diseases. GO enrichment terms showed inclinations to cerebral and neuronal constructions and functions. Transmission of nerve impulse (GO:0019226, P=2.8E-18), synaptic transmission (GO:0007268, P=5.1E-17), neuron development (GO:0048666, P=4E-13), neuron differentiation (GO:0030182, P=4.4E-13), cell morphogenesis involved in neuron differentiation (GO:0048667, P=8.6E-13), neuron projection development (GO:0048812, P=5.6E-12) and other GO terms related to the generation, function and neogenesis of neurons were significantly enriched. KEGG pathway analysis showed possible diseases involved of the DEGs, such as Amyotrophic lateral sclerosis (P=0.00022) and Dilated cardiomyopathy (P=0.0016). Neurotrophin signaling pathway (P=0.012) and Axon guidance (P=0.017) seemed to be highly related to ischemia cerebrovascular diseases.

The PPI network of model group versus XST group showed the gene-gene interactions between significantly and differentially expressed genes. To reveal deeper effects and mechanisms of XST on ischemic cerebral diseases, we compared the two sets of DEGs. There were 82 genes overlapped in the two sets of DEGs, GO enrichment analysis showed that phospholipid biosynthetic process (GO:0008654, P=0.0091), lipid biosynthetic process (GO:0008610, P=0.011) and phospholipid metabolic process (GO:0006644, P=0.046) were significantly enriched. Those DEGs varied divergently in the comparisons might elucidate how XST protect the ischemia brain.

ANTXR2, FHL3, PRCP and TYROBP are the 4 genes that up-regulated in blank group vs model group and down-regulated in model group vs XST group. ANTXR2 (anthrax toxin receptor 2) was originally identified as a result of up-regulation during capillary morphogenesis of endothelial cells (ECs) cultured in vitro [23]. Claire V. Reeves et al [24] used RNA interference technique to show that ANTXR2 and ANTXR1 functions as positive regulators of membrane type I-matrix metalloproteinases (MT1-MMP) activity [25], which is cellular collagenase essential for skeletal development, cancer invasion, growth, and angiogenesis [26]. FHL3 (four and a half LIM domains 3) is a member of the FHL protein family that plays roles in the regulation of cell survival, cell adhesion and signal transduction [27]. Angiogenin (Ang) is known to induce cell proliferation and inhibit apoptosis by cellular signaling pathways and by direct nuclear functions of Ang, Wenrong Xia et al [28] had identified FHL3 as a novel Ang binding partner. Their findings suggest that the Ang-regulated cell growth and apoptosis may be related to the interaction between Ang and FHL3. PRCP (prolylcarboxypeptidase) is associated with inflammation, suggesting it is a prerequisite for healing and scar formation [29]. Adams GN et al [30] had identified PRCP as a regulator of blood vessel homeostasis, the PRCP aortas express reduced levels of Kruppel-like factors 2 and 4, thrombomodulin, and eNOS mRNA, suggesting endothelial cell dysfunction. TYROBP (tyro protein tyrosine kinase binding protein) acts as a signaling adaptor protein for numerous cell surface receptors, playing important roles in signal transduction in dendritic cells, osteoclasts, macrophages, and microglia [31]. These 4 up-to-down genes might possibly be up-regulated by the MCAO related pathological process and down-regulated by the protective effects of XST.

Among the 23 down-to-up genes, TAF9B (TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor) is a subunit of Tfiid, which is vital in transcription initiation of RNA polymerase II preinitiation complex assembly [32]. Frontini M et al showed that TAF9B and TAF9 share some of their functions, and both TAF9 and TAF9B are essential for cell viability and transcriptional regulatory process. Research [33] has shown that TAF9B is selectively up-regulated in vitro motor neuron differentiation and is required for the transcriptional induction of specific neuronal genes. Fibroblast growth factors (FGFs) are polypeptides that are neuroprotective and can stimulate proliferation

of neural stem cells. Chadashvili T et al [34] had shown that FGFR2 (fibroblast growth factor receptor 2) might be involved in neurogenesis and the biological effects of FGF-2 is mediated through FGFR2-dependent mechanisms. Striatal medium spiny neurons (MSN) are critically involved in the coordination of movement, emotions, and cognition. Their degeneration would lead to several motional disorder diseases [35]. Arlotta P et al [36] found that BCL11B (B-cell CLL/lymphoma 11B) is pivotal to MSN differentiation, striatal patch development and the establishment of the cellular architecture of the striatum. Meanwhile Tang B et al [37] reported that BCL11B is a novel regulator of the brain-derived neurotrophic factor (BDNF) signaling pathway, which is disrupted in many neurological disorders. RB1CC1 (RB1-inducible coiled-coil 1) is a crucial signaling component to coordinately regulate different cellular processes since it interacts with multiple signaling pathways [38]. It is required for the maintenance and differentiation of postnatal neural stem cells [39]. The down-regulation of RB1CC1 would leads to cerebellar degeneration caused by increased neuronal death [40] and axon degeneration and neuronal atrophy through mTOR signaling alteration [41]. MBNL2 (muscleblind-like splicing regulator 2) is a member of the family of Muscleblind RNA binding proteins that are known to bind CTG/CCTG RNA repeats. Current researches about MBNL2 show its significant affects in the pathological features of myotonic dystrophy [42, 43]. MBNL2 may be related to body numbness and paralysis after stroke, yet there is no direct evidence of it. These down-to-up genes mentioned above are reportedly neuroprotective or essential to the neuron generation and neogenesis in certain aspects. They were down-regulated after MCAO model was established and significantly up-regulated after XST intervention. These genes might possibly be the protective sites of XST.

Based on existing researches, these DEGs varied divergently in the comparisons jointly showed how the XST protects the brain and neurons in genomic level, including regulation of angiogenesis, vascular morphology, neuronal generation, differentiation and neogenesis, neuron signal transduction and neurotrophic factors. Interestingly, those protective mechanisms of XST from the prospective of genomics have identical views with Traditional Chinese Medicine. In the prospective of TCM, XST treating ischemia vascular disease with the function of promoting blood circulation and removing blood stasis. In that could dissolve the thrombosis and nourish the ischemic organ and tissue. These findings have given great inspirations to the exploration of therapeutic mechanisms of Chinese medicine and the modern scientific illustrations of TCM.

Conclusion

To summarize this research comprehensively, MCAO is a pathological process involving multi-aspects, such as cell cycle, apoptosis, hemorrhagic transformation, neurotrophin regulation. Identifying the pivotal biological and pathological process related to cerebral ischemia diseases could contribute to better treatment and prognosis. XST as a preparation of Chinese medicine extracted from medical herbal, has explicitly protective effects on ischemia cerebrovascular diseases. And the therapeutic mechanisms are illustrated via genomic and bioinformatic technology. The identified therapeutic mechanisms resemble the treatment of TCM. The new interpretation of TCM and Chinese herbal is urged.

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Disclosure of conflict of interest

None.

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