The Network Regulation of Yiqi Jiedu Formulae Against Cerebral Ischemia Based on Central-periphery Inflammation System

Ruocong Yang
China Academy of Chinese Medical Sciences
tingting Zhao
China Academy of Chinese Medical Sciences
Liming Liu
China Academy of Chinese Medical Sciences
Shaojing Li (shaojingli2004@126.com)
China Academy of Chinese Medical Sciences

Research Article

Keywords: Inflammation，Gene-network，Formulae，Central-peripheral，Hub-genes，Stroke，mRNA

Posted Date: June 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-583166/v1

Abstract

Background: Connections between inflammation and gene-network regulation are suggested important in understanding the therapeutic target of stroke and in illuminating underlying mechanism. However, studies on the establishment of network relating with inflammation during stroke process are still in their early stages.

Results: Herein, Message RNA chips were used to scan whole genome of stroke-model rats. We selected the inflammation genes from the whole mRNA expression results. And after a series of analysis, we tried to establish a central-peripheral inflammation network on MCAO rice which then be used as disease background network for our further study. As for the background network, we found and verified some key node genes (also named as hub-genes), which are joint in several inflammation and immune related pathways. While mapping genes from treatment group to the background network, we found the promising target genes of Yiqi Jiedu formulae, a traditional chinese prescription used in clinic for stroke, which might give an explanation to the common characteristic about TCM treatment called PK-PD inconsistent.

Conclusions: The mRNA network-based analysis provides a foundation for elucidating inflammation-disease associations, a rather promising insight into the inflammation progress during stroke and a novel strategy to reveal the underlying mechanism of TCM.

Background

Traditional Chinese medicine (TCM), an ancient therapeutic system, has been used in China for more than thousands of years. As one of the most important and effective treatment, formulas were commonly used to cure diseases. In clinic daily practice, Yiqi Jiedu Formula (YJ) shares good effect to treat cerebrovascular diseases. In our previous studies, the significant anti-ischemic stoke effect of the new combination drug, YJ, derived from the above homonymous Chinese medicine, which consists of *Panax ginseng*, *Rhizoma Coptidis* and *Gardenia jasminoides*, has been verified in middle cerebral artery occlusion (MCAO) model rats[1]. However, just like this formula, the explanation of the common phenomenon of TCM for how to treat cerebrovascular diseases when the main therapeutic components always have low bioavailability and even hardly transmit Blood Brain Barrier (BBB) to reach damaged region, which we named Pharmacokinetic(PK)-pharmacodynamic (PD) inconsistent, remains unclear. This is one of the main as well as crucial obstacle to clarify the treatment role and function mechanism of TCM.

Normally, the homeostasis of the central nervous system is maintained by the blood-brain barrier (BBB). BBB was regard as a transmit limiter that could control the transport material into brain regions. The brain has traditionally been considered as an immune privileged organ, a distinct and prominent immune system in the body. However, new brain lymphatic vessels drain old concepts, the evidence for lymphatic vessel lymphatic vasculature in brain establish the direct relationships between the CNS and the immune

system,immune cell trafficking into the CNS during immunosurveillance and neuroinflammation. However, with the discovery of lymphatic vessels in central nervous system (CNS), the view has been drastically changed[2]. After that, some researchers further reported that there might be an underlying relationship between brain and peripheric parts, in some ways, messaging by some specific substance or transmission signals[3–5]. More interestingly, senses of co-ordinate expression of specific mRNAs were found in both brain region cells and those in peripheric organs.Which might be regarded as one of promising message carrier that transfer bio-information into brain and activate the associated signal pathway across BBB [6, 7].

The discovery of the CNS lymphatic system may call for a reassessment of basic assumptions in neuroimmunology and shed new light on the etiology of neuroinflammatory and neurodegenerative diseases associated with immune system dysfunction Structural and functional features of central nervous system lymphatics spatially and temporally coordinate centraland peri-immune system connections may have a profound physiological meaning.

Based on above, in this paper, we proposed a central-peripheric inflammation regulation network to elaborate the phenomenon of PK-PD inconsistent of YJ as a Sample. MRNA gene microarray was designed to analysis the expression on mRNA levels of different organs(brain,spleen□blood), and to establish background gene map of stroke. To explain the mechanism of YJ to protect from inflammation damage during stroke pathological process, inflmmatory genes were selected as therapeutic targets and network was chosen as methodology.

Results

1.1 Neurological function score

Neurological function score is an index to judge the damage of ischemic stroke [8]. The results of scores were listed in Fig. 1. Compared with sham groups, rats in model group showed significant changes both on movements and neurological scores ($P < 0.01$) after operation. The situation was different in YJ group. Though behaviours of YJ group rats were not as good as those ones in model group, it is significantly better than rats in model group, and the effectiveness of YJ is also clearly exhibited via neurological scores ($P < 0.01$).

1.2 Microarray statistics

To investigate genes expression profiles that are associated with the pathologicalMCAO model, mRNA expression in different organs (brain, spleen and blood), in both 6h and 8h in sham rats and MCAO model rats, were profiled using gene chip. Total number of 1822 genes in 6h and 3146 were identified that showed statistically significant differential expression in MCAO model rats when compared to sham ones (fold change value>2 or fold change value<0.5, meanwhile, P-value<0.05). To clearly show the distribution

of these genes, Venn diagrams were used to present the results classified in different times. As a result, for 6h, 1099 genes were expressed differently in brain (including 59 geneswere same with spleen, 21 with blood, and 8 with both spleen and blood); 624 genes of spleen showed different expression (including 59 were same with brain, 48 with blood, and 8 with both brain and blood); 242 genes in blood were expressed differently (including 48 were same with spleen, 21 with brain, and 8 with both brain and spleen). For 8h, there were 536 genes expressing differently in brain (including 75 were same with spleen, 14 with blood, and 4 with both spleen and blood); a total number of 2355 gene was expressed differently in spleen (including 75 were same with brain, 121 with blood, and 4 with both brain and blood); 473 genes differently expressed in blood (including 14 were same with brain, 121 with spleen, and 4 with both brain and spleen). When putting all the different genes together, the data went as following. Count number of 1375 genes were found differently expressed in brain (including 276 were same with spleen, 49 with blood, and 32 with both spleen and blood); a set of 2892 gene were different in spleen (including 276 were common with brain, 206 with blood, and 32 with both brain and blood); a number of 691 genes expressed differently in blood (including 49 were same with brain, 206 with spleen, and 32 with both brain and spleen). The summary result shown as Venn figure were present in Fig. 2.

1.3 Enrichment and network establishment of differential-expression genes

GO analysis and pathway analysis were applied to classify the data of differentially expressed genes. As a result, for GO analysis, there are 105 biological processes in 6h and 26 ones in 8h, while the counts of pathway analysis are 80 in 6h and 123 in 8h respectively. Among these GO processes and pathways, most of them were related with inflammation process, and the genes involved in these processes and pathways were generally classic but crucial inflammation-related genes, such as Mapk9 in MAPK pathway, Nf-κb1 and Rela in NF-κB pathway, Jak2 and Jak3 in Jak-STAT pathway. These analyses indicated that inflammation was one of the main characters in stroke process. Then the different genes were put into KEGG database to establish networks for 6h, 8h and whole differential genes. The results were presented in Fig. 3.

1.4 Analysis of inflammation related genes

To further investigate and establish the inflammation regulation network as well as the relationships between inflammation related genes during stroke pathological progress, all genes related with inflammation were selected for next study. As a result, a total number of 149 inflammation related genes were found to express differently in MCAO rats when comparing with those in sham group. When putting these genes in Go analysis, 51 biological processes were enriched in 6h and 18 ones were collected in 8h, while the counts for pathway analysis are 68 in 6h and 71 in 8h respectively. When enriching all the differential genes both in 6h and 8h, 67 biological progresses and 94 pathways were regulated as a

result. Finally, the different genes related with inflammation were put into KEGG database to establish networks for 6h, 8h and whole differential genes. The results were presented in Fig. 4.

1.5 Certification for the inflammation network by RT-PCR

To further certificate the veracity of our established network, 21 hub-genes in total were selected to confirm the expression trend by RT-PCR compared with the ones showed in gene chips. The confirmation genes were listed in Table 1. In gene chip test, there were 19 genes showed up-regulation and 2 genes presented down-regulation in the comparison between sham group and model group, while 16 genes were up-regulated and 2 genes were down-regulated in PCR test. As a result, there were 18 verified genes were in same expression trend with the data in the network. For these result, the works of analysis for progress and the establishment for inflammation network could be considered reliable. The results were listed in Fig. 5.

Table 1
the confirmation gene list by Real-time PCR

Number	Gene name	Gene ID
1	Ccl20	20297
2	Ccl3	20302
3	Ccl7	20306
4	Cxcl1	14825
5	Cxcl16	66102
6	Lyn	574428
7	Socs3	17096
8	Stat3	12702
9	Jun	20848
10	Nfkbia	16476
11	Fos	18035
12	Eif2ak1	14281
13	Ccr5	15467
14	Cx3cr1	12774
15	Cxcr6	13051
16	Pik3r3	80901
17	Ptk2b	18710
18	Stat5a	19229
19	Rora	20850
20	Il18r1	19883
21	Ccr6	16182

1.6 Regulations between CNS and peripheral organs

To better understand whether there exist interactive-regulations between CNS and peripheral parts during 6h to 8h, relation built of CNS's and peripheral genes was further designed. Genes of brain in 6h and the ones in spleen and blood in 8h, as well as the genes of spleen and blood in 6h and the ones of brain in 8h were collected for further study. Two networks were composed to present the regulation between CNS

and peripheral organs. As the result, several classic inflammation genes were found acting as key genes during the period of 6h to 8h after ischemic stroke operation. Among these genes, we further circled the inflammation related one with a blue ring outside. These inflammation related ones could be mainly classified into three families/pathways: apoptosis related pathways, chemokines family and PI3K-Akt pathway. In addition, from these distribution of organs and time point genes belonging to, it is gradually clear that there indeed exists strictly sequential inter-regulation between CNS and peripheral organs. The two established networks were presented in Fig. 6.

1.7 Regulation of YJ to the inflammation network

With the accomplishment of background network, the mechanism of YJ were designed as final step. Gene data of YJ group were enriched and mapped on the inflammation network of whole inflammation related genes we got in 3.4 (Fig. 4C). As a result, a total number of 67 genes were found as hub genes enriched in four main pathways, including NF-kappa B pathway, PI3K-Akt pathway, Chemokine pathway, Jak-STAT pathway. These results indicated that YJ could prevent damages caused by inflammation during stroke pathological process, and genes related with apoptosis related pathways, chemokines family and PI3K-Akt pathway might be promising therapeutic targets. The results were presented in Fig. 7.

Discussion

The phenomenon of PK-PD inconsistent is common in TCM studies and clinical practices[9, 10]. Although drug combination might improve permeability across BBB and increase the concentration of effective components from TCM in brain[11], there are still so many intractable problems that the therapeutic substances share good treat effect with little compounds, besides, their metabolic pathways in damage regions are still undefined. Researchers have provided some solutions that aim to illustrate these confusing facts, however, there is still no convincing hypothesis. Thus, to try to settle with this issue, an innovative thought in our present study was proposed after a series of tests. In our hypothesis, it is believed that for some traditional Chinese medical herbs and therapeutic effective parts from them, instead of directly treating on diseased regions, these effective compounds may target on other organs or parts of organisms. To prove it, YJ, an effective formula in clinic to prevent damage during ischemic stroke, was used as an example.

Stroke is the leading cause of death and permanent disability worldwide[12]. With increasing evidences shown, although the detailed mechanisms are largely unclear, inflammation is noticed to act importantly in the progression of ischemic stroke[13–15]. Both pre-clinical stroke studies and clinical studies indicate that inhibition of inflammatory responses can effectively decrease brain injury and improve neurological outcome[16–20]. As the whole formulae was used to protect organisms from stroke damage and two main components of YJ were considered to share good effect on anti-inflammation [21, 22], inflammation and related methodology were conducted in this study.

Since therapy target of TCM are too massive and it is the difficult to research in inflammation without a wide view of the whole map, network is designed as main method in this study to cover this problem. Considering that mRNA chips have been well applied in biological studies, and combining it with method of network establishment can clearly and systematically demonstrate the underlying regulation effects of traditional Chinese medicine on mRNA level, we combine these two methods as our tools to establish gene networks in our study.

For a long time, CNS is always considered as a relatively independent system which is blocked by brain-blood barrier. However, with the presence of a functional and classical lymphatic system in the central nervous system were found in 2015[23], current common view that regarded brain tolerance and the immune privilege of the brain should be revisited. Basing on these facts, studying on regulation of inflammation and relationships between CNS and peripheral system during stroke was reliable. For CNS, we chose brain, the most inflammation related organ in CNS[24, 25], as the investigated subject for CNS. Meanwhile, spleen, the organ that is crucial and responsible for both innate immune and acquired immune[26, 27], was selected as the delegate for peripheral system. And to connect the CNS and peripheral part in the network, the blood was separated as bridge to connect brain and spleen.

As result of our study, three pathways/factor families, including chemokines pathway, PI3K-Akt pathway and apoptosis related pathways. were found have important roles in stroke inflammation process. In Fig. 4, we can easily summary these scattered genes into these pathways. Chemokines family is a family of small cytokines, or signalling proteins secreted by cells, and it always plays an important roles during inflammation inducement[28, 29]. These cytokines located in the up-stream of inflammation process, inducing the start of inflammation then the damage occurs. In our study, Il-1, Il-6 and other chemotactic factors were remarkable in the background network. PI3K-Akt pathway, as an intermediate transit point, passes bio-signals to down-stream pathways[30, 31]. In this study, mainly MAPK pathway and NF-κB pathway were involved in this signal passing process. Apoptosis related pathways, including Jak-STAT pathway, MAPK pathway and NF-κB pathway in our study, is the major damaged effect inducing by stroke. Signals passing in these pathways, mainly expressed in brain samples as noticed in Fig. 4, directly lead brain cells to death. In addition, we as well observed the dynamic signal transduction in Fig. 6. We dynamically noticed the signal transmit between CNS and peripheral part, and the signals belonging to chemokines family were the key nods in the inflammation process.

As shown in Fig. 7, therapeutic effect on stroke of YJ Formulae were focused on chemokine pathway and apoptosis related pathways (including Jak-STAT pathway, MAPK pathway and NF-κB pathway). As to hub-genes, four hub-genes were regarded as the most crucial ones in the regulation networks: Tnf in TNF pathway, Cxcr5 in chemokine pathway, Jak2 and Socs-2 in JAK-STAT pathway, and NF-κB1 in NF-κB pathway. These four genes have already been proved remarkable in stroke process. Respectively, it is evidenced that Tnf is involved in every phase of stroke-related neuronal damage, especially in early phase of this disease[32]; it is proved that Jak and Socs-2 take part in the stroke pathologic process via the JAK-STAT pathways[33, 34]; and NF-κB is one of the most critical factor in nuclear that is responsible for DNA damage process and has been a promising therapeutic target for ischemic stroke[35]. Thus, according to

our results and current studies, YJ, especially the components which cannot transmit through BBB, might prevent from stroke by regulating these hub genes and their related ones. The possible mechanism is to regulate the chemokines expression in spleen, then translate these factors via blood into brain, and finally to take effect in brain.

Materials And Methods

1.8 Animals

This study was performed according to the Guide for the Care and Use of Laboratory Animals, which was published by the US National Institutes of Health (National Institutes of Health Publication No. 85 – 23, revised 1996) and was approved by the Ethics Committee of China Academy of Chinese Medical Sciences. Adult male Sprague-Dawley rats weighing 230–250 g were obtained from the Animal Breeding Centre of Beijing Vital River Laboratories Company (Beijing, China). All animals were housed individually at 22 ± 2°C with a relative humidity of 50 ± 10% and a 12-h light/12-h dark cycle. The animals were kept in a pathogen-free environment with free access to food and water. The experimental procedures were approved by the China Academy of Chinese Medical Science's Administrative Panel on Laboratory Animal Care.

1.9 Materials

Berberine (Purity ≥ 95.18%) was purchased from Xian yang Aviation 168 Bio-engineering Co., Ltd (Xian yang, China). Jasminoidin (Purity ≥ 99.68%) was purchased from Baoji Fangcheng Bio-engineering Co., Ltd (Baoji, China).Ginsenosides (Rg + Re + Rd ≥ 40.55%±5%) was purchased from Nanjing Zelang Co., Ltd (Nanjing, China). EGb761 was purchased from Dr. Willmar Schwabe (Karlsruhe, Germany, 4250213).

1.10 Animal models and experimental design

After 48h of acclimatization, the rats were anesthetized with chloral hydrate at a dose of 400×10^{-3} g•kg^{-1} (i.p.). The rectal temperature was recorded and maintained at 37 ± 0.5°C throughout the surgical procedure. The MCAO operation by the intraluminal filament method was performed according to a previously reported method with some modifications[8]. Two timepoints, 6h and 8h were chosen after MCAO operation in this study,. The rats were randomly divided into 6 groups according to a uniform design (n = 3/group): 6h sham group, 6h vehicle control group, 6h YJ groups (25mg•kg^{-1}), 8h sham group, 8h vehicle control group, 8h YJ groups (25mg•kg^{-1}). The sham operation group was subjected to only preoperative anesthesia and blood vessel separation; the other groups were subjected to the MCAO model. After 6h, neurological function score was assessed, then the animals were sacrificed to obtain brain, spleen and blood respectively. Furthermore, brains and spleens were made as homogenate and white blood cells were separated from whole blood for further tests.

1.11 Microarray analysis

RNAs of the three parts of each rat were extracted with Qiagen RNA kit. Total RNA was reverse transcribed, amplified, labelled and hybridized to Rat Genome 1.0 arrays (Affymetrix). Microarray data sets were analysed with Agilent Genespring GS 11 software. RNA of different samples was prepared in the same procedure and used in microarray analysis.

1.12 Investigating of differential expression genes

Genes were standardized and interpreted functionally before comparison. Using RVM t-test (random variance model t-test) and the short-survival group as the control group, the P value and the false discovery rate (FDR) were calculated for each differential expression gene. FDR was calculated to correct the P-value, which controls type I errors. With a threshold of P value < 0.01 and FDR < 0.05, survival duration-related differential expression genes were picked out. The up-regulation genes and the down-regulation genes can be classified by the fold change of intensities

1.13 Enrichment analysis

1.13.1 Gene ontology (GO) analysis

Based on Gene Ontology Database (http://www.geneontology.org/) and UniProt Database (http://www.uniprot.org/), the significance level of GOs of the survival duration-related differentially expressed genes was analysed by two-side Fisher's exact test and 2 test. The differential expression genes were analysed independently according to up- and down-regulation of these genes. We computed P-values for all the differential expression genes in all GO categories, and the threshold of significance was defined as P-value < 0.01 and FDR < 0.05. Each GO was also analysed by enrichment analysis using the following formula: Re = (nf/n)/(Nf/N), where nf refers to the number of differential expression genes within the particular category, n to the total number of genes within the same category, Nf to the number of differential expression genes in the entire microarray, and N to the total number of genes detected in the Microarray. Matlab (http://www.mathworks.com) and Mysql (http://www.mysql.com/) were used as the analysis platforms.

1.13.2 Pathway analysis

Data of signaling pathways were from KEGG (http://www.genome.jp/kegg/), Biocarta (http://www.biocarta.com/), Humancyc (http://humancyc.org/), Reactome (http://reactome.org/) and NCBI Database (http://www.ncbi.nlm.nih.gov/). All signaling pathways were analysed for the significance level, using P < 0.01 and FDR < 0.05 as the threshold, Matlab and Mysql as the analysis platforms. The enrichment was calculated like the equation above.

1.14 Dynamic gene network analysis

The normalized signal intensity of significant differential genes was used to build a co-expression network. At first, the Pearson's correlation of each pair of genes was calculated as the basis of choosing the significant correlation gene pairs. Then the gene-gene interaction network was established according to the correlation between genes. Within the network analysis, nodes represent the genes, and the edges between genes depict the interaction between them. All the nodes were marked with degree, which is defined as the link numbers one node has to the other. Genes with higher degrees occupied more central positions in the network and had a stronger capacity of modulating adjacent genes. In addition, k-core in graph theory was applied to describe the characteristics of the network including, but not limited to, the centrality of genes within a network and the complexity of the sub-networks. According to the relationship between genes, they were divided into several sub-networks, and marked with different colours.

1.15 Validation of microarray data by Real-time PCR

To confirm the reliability and facticity of the background network, we chose 21 genes from different organs as the test genes. Three parameters, degree (the total inward and outward connections of one gene), fold change value (the ratio of sham group to model group) and p-value (the expression difference between sham group and model group) in detail respectively, were considered during the choose process. The threshold of degree is set greater than 3 folds, the fold change value is over 1.3 folds, and the p-value is under 0.05. Total RNA was extracted from sample organs as used in microarray assay including three independent experiments Trizol Reagent (Life Technologies). Following purification with an RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's manual. M-MLV reverse transcription (Promega) was used to synthesize cDNA. Quantitative PCR analysis and data collection were performed on the ABI 7900HT qPCR system using the primer pairs listed below. The raw quantifications were normalized to 18s values for each sample and fold changes were shown as mean ± SD in three independent experiments with each triplicate.

1.16 Treatment group on inflammation network

Treatment group data were mapped onto the established inflammation networks (established in 2.7). Different colours filled in circle stood for the sources from different organs.

Conclusions

In conclusion, in this study, we proposed a novel strategy to elaborate on the common phenomenon of PK-PD inconsistent in TCM, and as a result, the presence inflammation networks connecting CNS and peripheral system was confirmed by detecting mRNA expression and taken YJ formulae as an example.during the pathophysiological stroke process and it is an underlying mechanism for the components with features of low bioavailability of YJ to take effect on treating diseases. For further

studies, our study on stroke, combined with central nervous system inflammation system in CNS and the periphery immune system as well as the illustration of the mechanism to YJ, may provide the basic information for further pathogenesis on this disease and other inflammation related illnesses, and the genes in established networks could be therapeutic targets for other promising drugs.further studies will be necessary to fully assess and characterize.

Declarations

Authors' contributions

S.J.L. conceived and designed the experiments as the corresponding author. R.C.Y., Z.T.T., and L.L.M., performed the experiments. R.C.Y. collected, analyzed and interpreted the data. R.C.Y. wrote the manuscript. All authors read and approved the final manuscript. S.J.L. supervised the study.

Author details

[1] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China.[2] School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China.

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study was carried out according to the Regulation on the Administration of Laboratory Animals published by the Ministry of Science and Technology of the People's Republic of China. All operations in the experiment followed the regulations of the Beijing Municipal Laboratory Animal Ethics Committee and were approved by the Animal Ethics Committee of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. This study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

Raw sequence data for this study are reliable and can be reasonable request.

Funding

This study was supported by National Key R&D Program of China (Grant no. 2017YFC1701900), and the Major Scientific and Technological Special Project for “Significant New Drugs Creation” (Grant no. 2019ZX09201004,).

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Figures

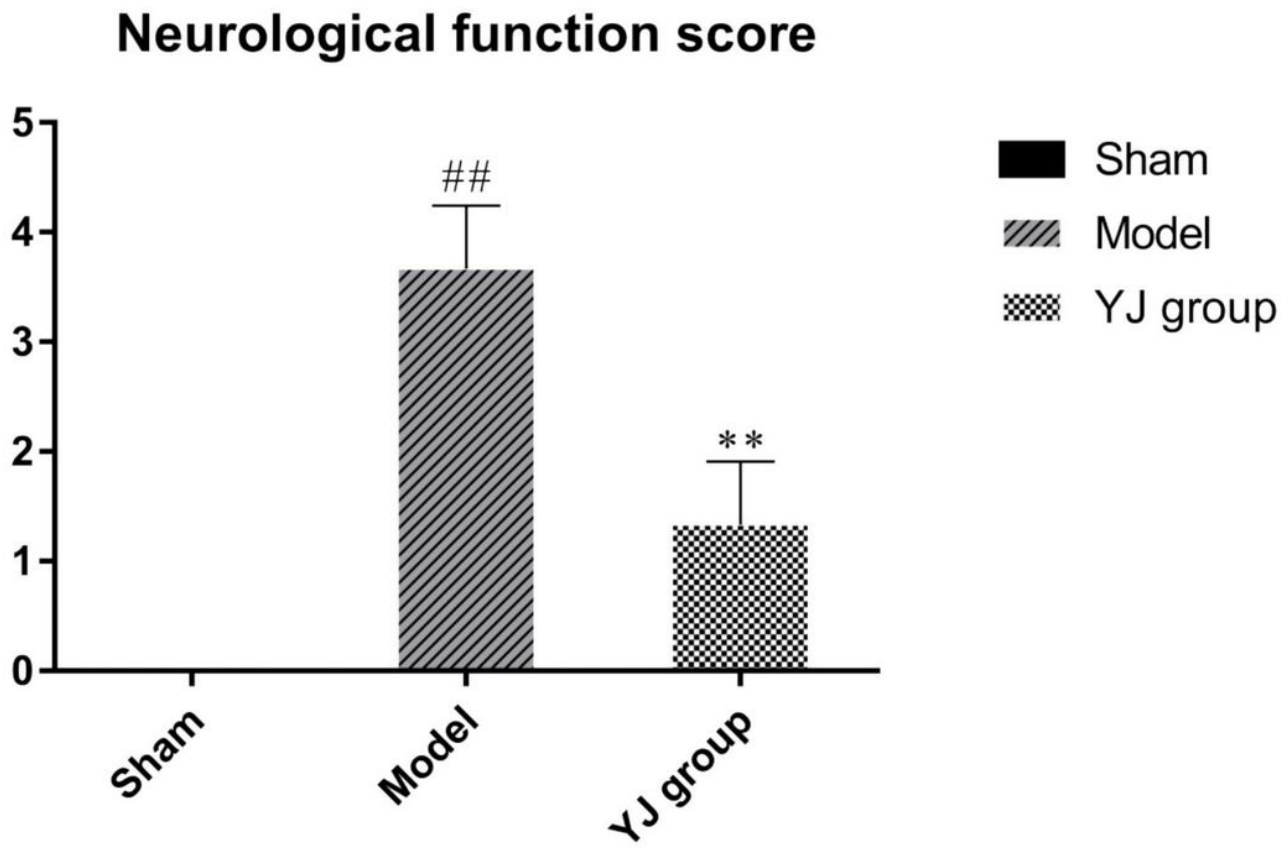

Figure 1

Effects of YJ on Neurological Score of rats after MCAO. Compared with shame group, ##P<0.01; compared with model group, **P<0.01.

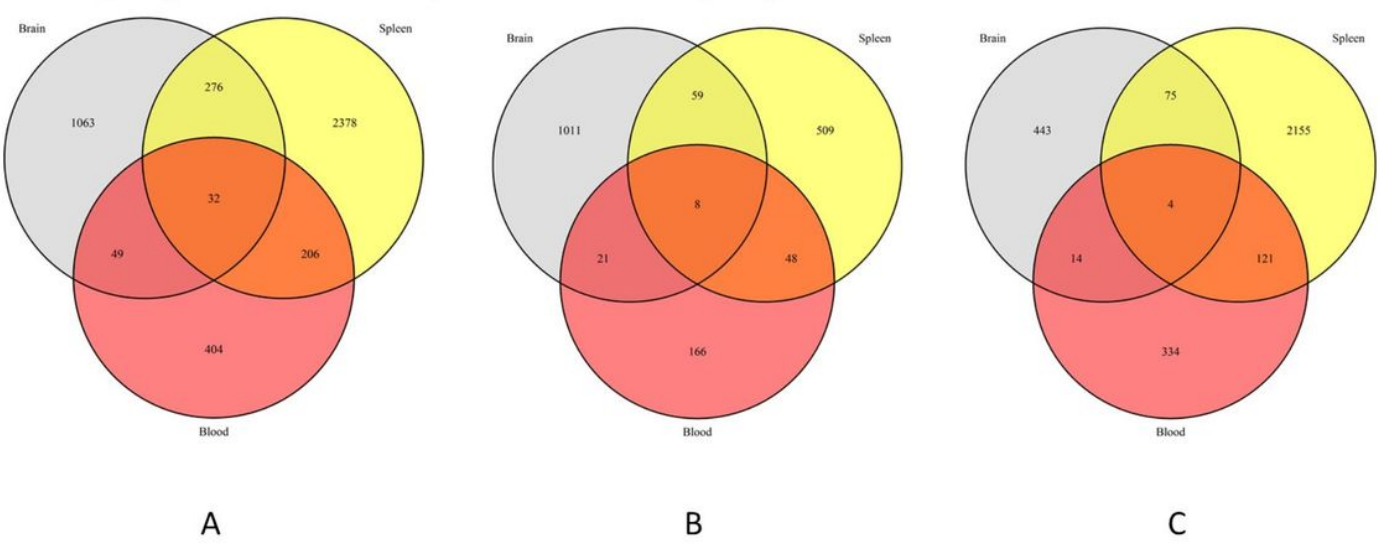

Figure 2

Venn diagram of organs. Venn diagram is used to present the distribution in organs of differential expressed genes at 6h and 8h. (A) the whole genes of the three organs, including 6h and 8h. (B) the genes of 6h. (C) the genes of 8h.

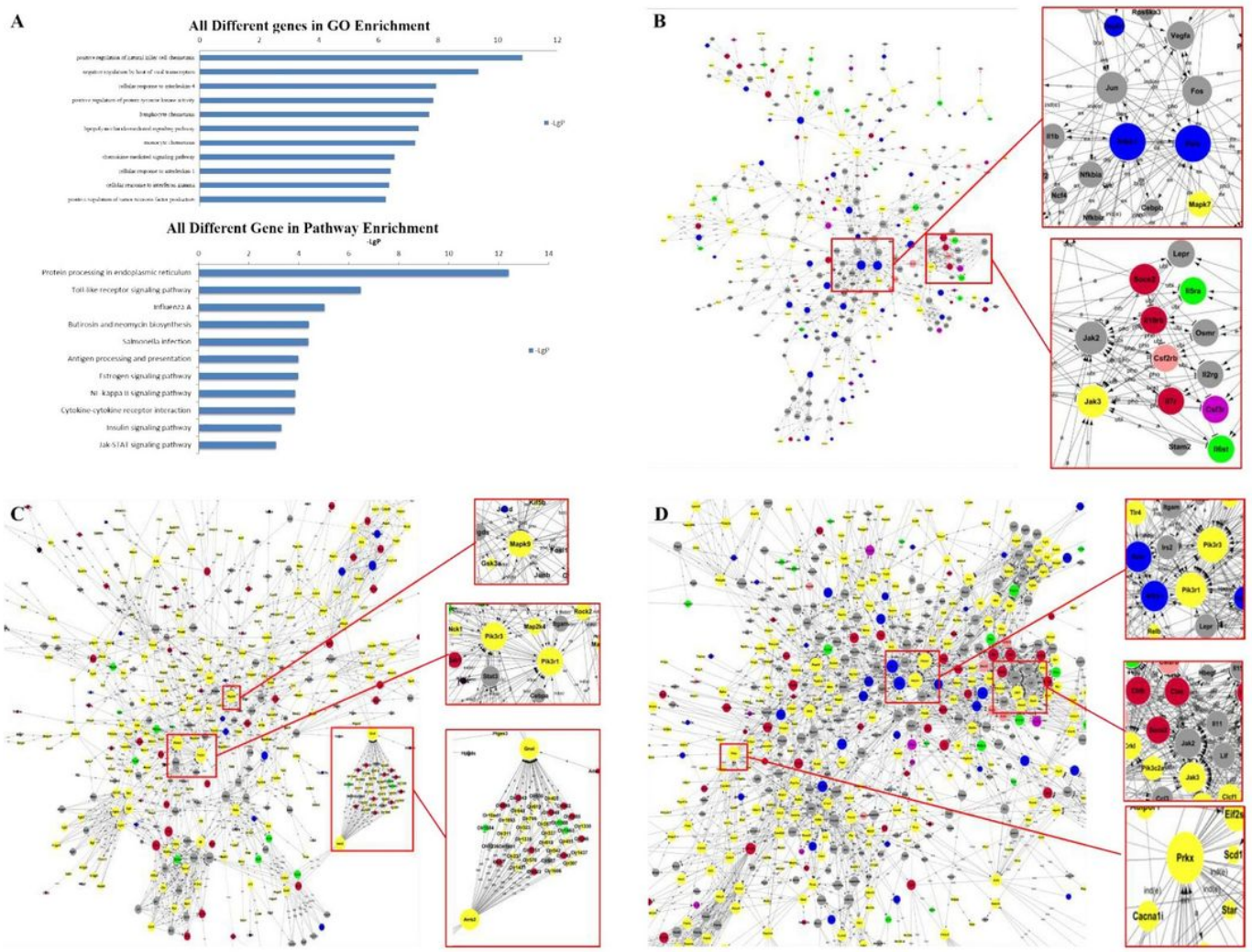

Figure 3

A. the results of Go and pathway analysis of all the genes showed significant differences. B. the network presenting the relations of the differential genes in 6h. C. the network presenting the relations of the differential genes in 8h. D. the network presenting the relations of the differential genes in both 6h and 8h. In this figure, different colours were used to represent the genes expressed in different organs. Genes coloured in grey refer to the ones expressed in brain, similarly, red for blood and yellow for spleen. Different from the genes expressed in single organ, some genes expressed synchronously in different organs. Genes in blue stood for those showing significant difference, comparing with sham groups, both in brain and spleen, while purple for those in brain and blood, green for those in spleen and blood, and pink for the ones expressed in the whole three organs.

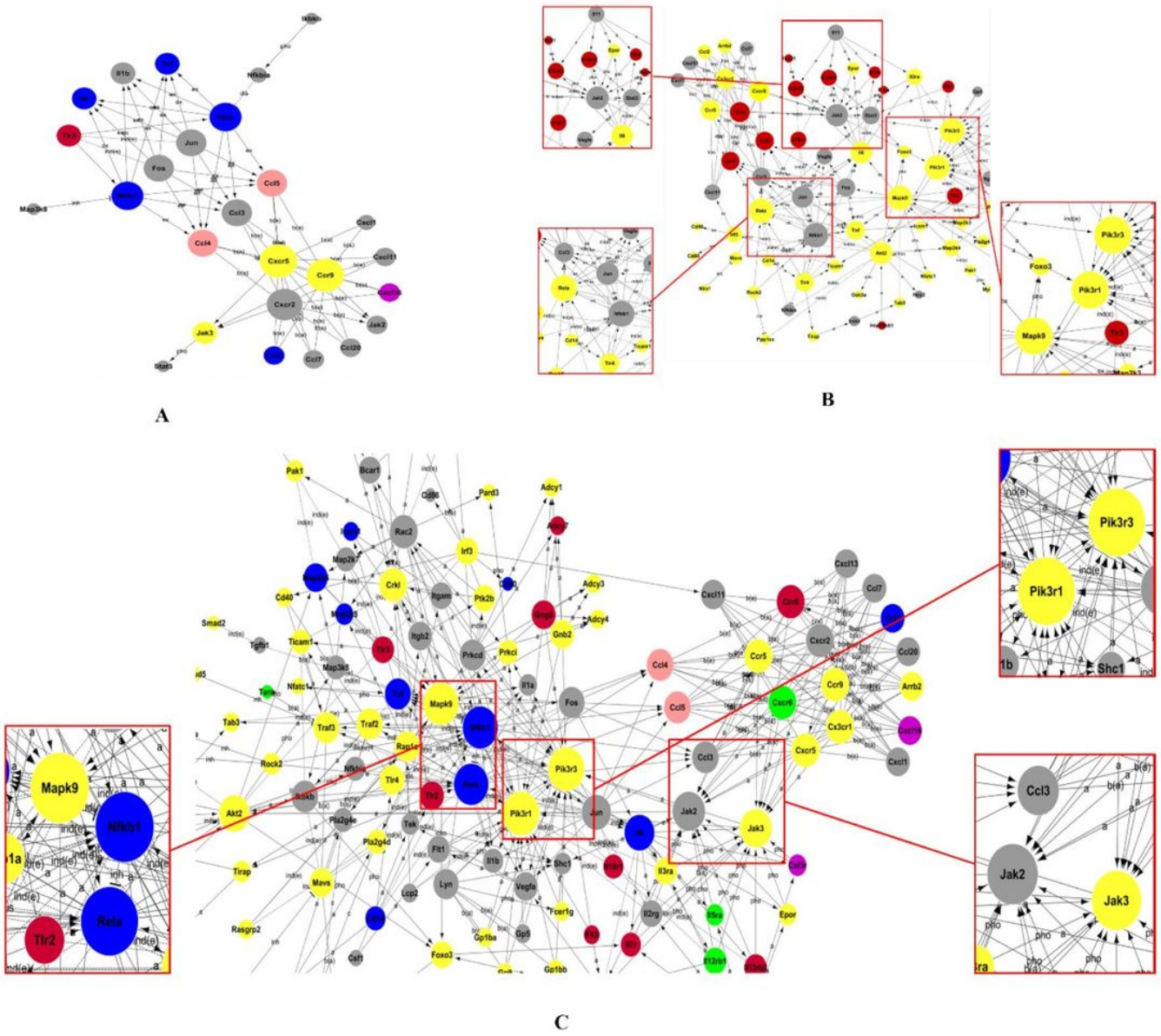

Figure 4

A. Inflammation network of 6h. B. Inflammation network of 8h. C. Inflammation network of all selected inflammation related genes. In this figure, different colours were used to represent the genes expressed in different organs. Genes coloured in grey refer to the ones expressed in brain, similarly, red for blood and yellow for spleen. Different from the genes expressed in single organ, some genes expressed synchronously in different organs. Genes in blue stood for those showing significant difference, comparing with sham groups, both in brain and spleen, while purple for those in brain and blood, green for those in spleen and blood, and pink for the ones expressed in the whole three organs.

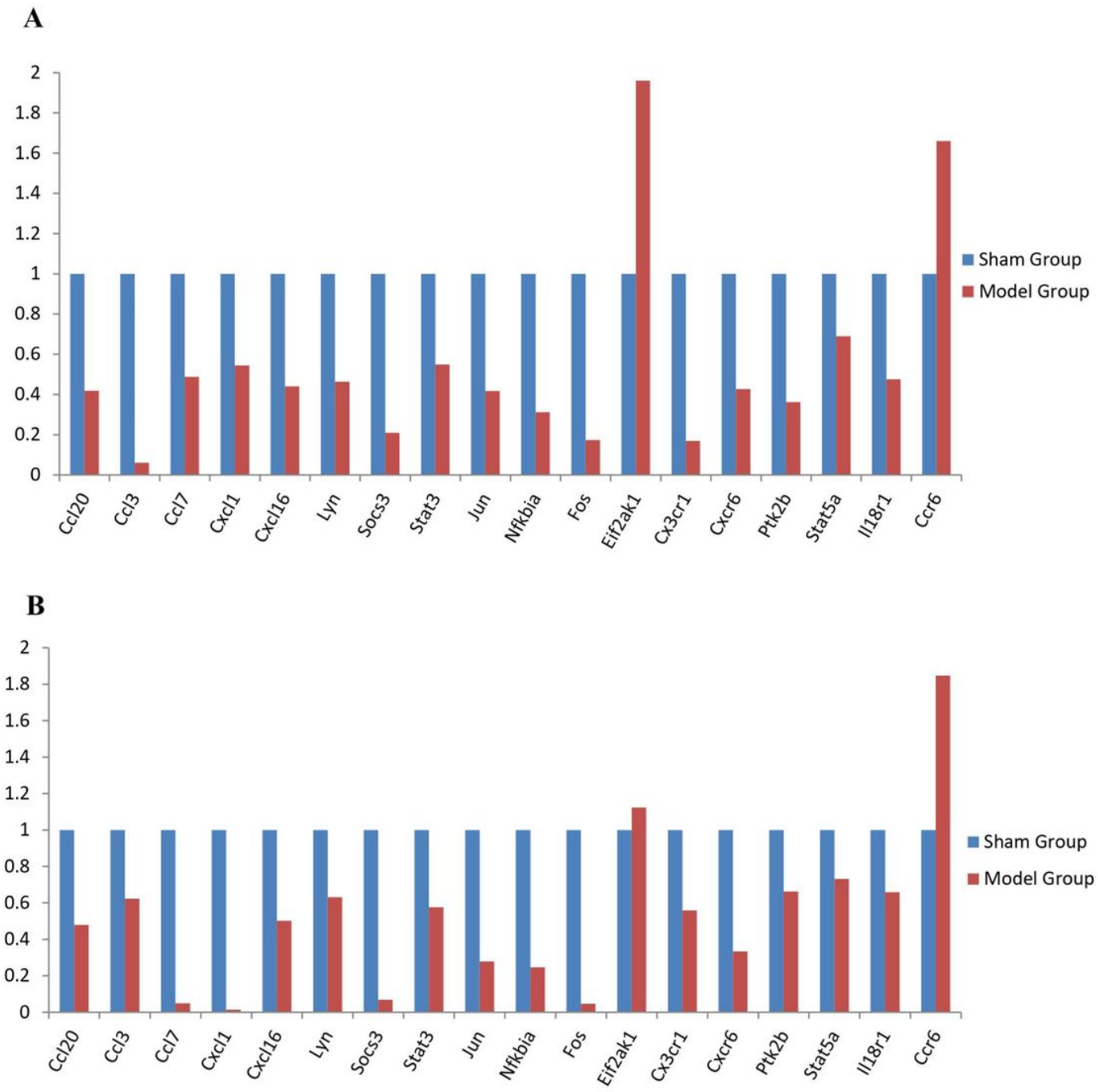

Figure 5

A. Results for verified genes in mRNA. B. the results of PCR test. Comparing with data from mRNA, PCR test showed good characters of high homogeneity. In all verified 21 genes, 18 genes in PCR test showed same expression trend with the data in network.

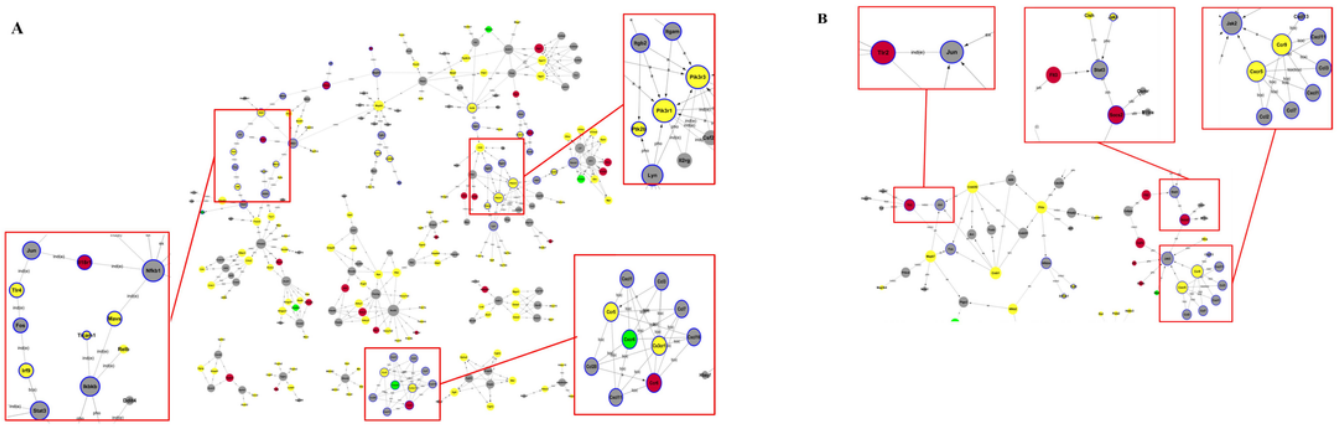

Figure 6

the regulation to stroke damage between CNS and peripheral. A. the positive trend regulation of CNS organ (brain) to peripheral organs (spleen and blood). B. the negative trend regulation which is from peripheral organs (spleen and blood) to CNS organ (brain), responding for stroke damage during 6h to 8h period. Similarly, genes in different colour stand for the different sources from different organs. In detail, grey refer to the ones expressed in brain, red stands for those from blood and yellow means spleen. For synchronously expression in different organs, genes in blue stood for those showing significant difference, comparing with sham groups, both in brain and spleen, while purple for those in brain and blood, green for those in spleen and blood, and pink for the ones expressed in the whole three organs.

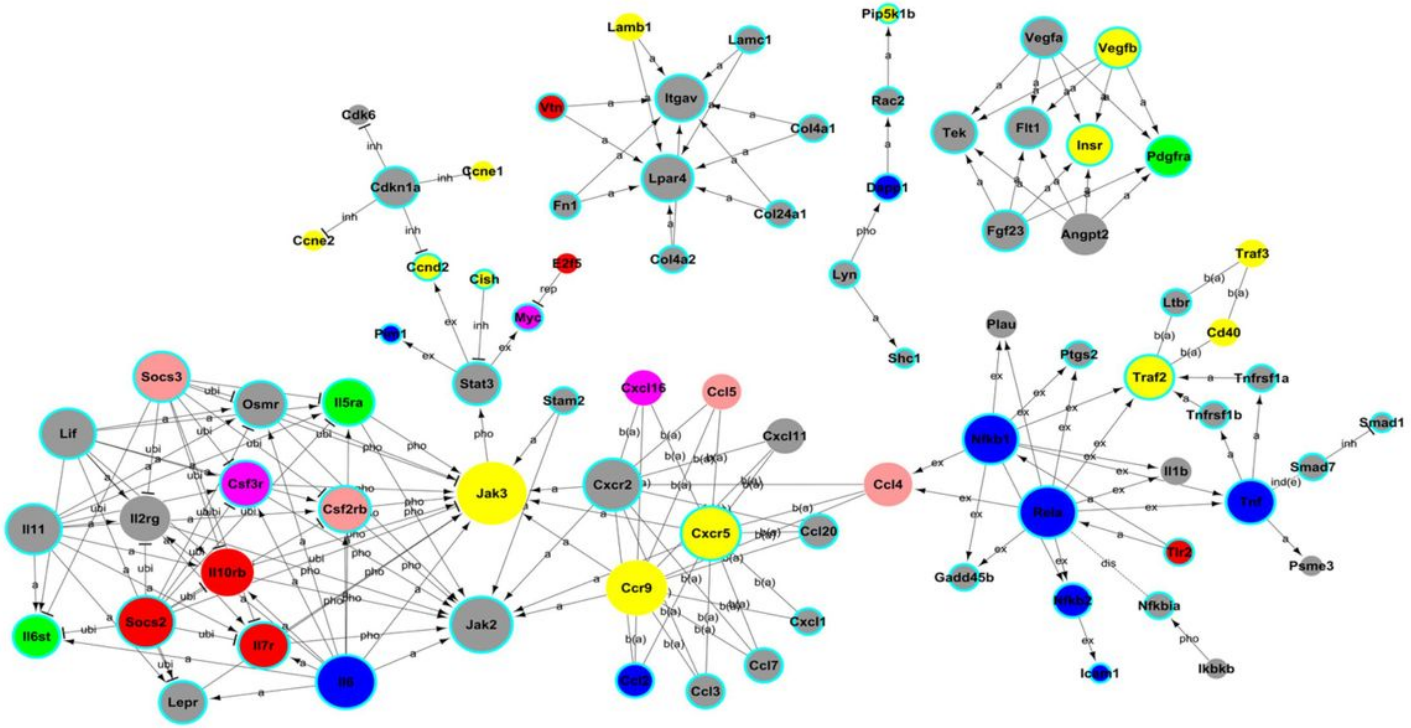

Figure 7

treatment target of YJ formula on inflammation network. Like meanings in last figures, colours are in same implication as the former ones, namely, grey for brain, red for blood, yellow for spleen, blue for brain and spleen, purple for brain and blood, green for spleen and blood, and pink for the whole three organs. Genes with cyan circle outside are those as therapeutic targets in stroke pathologic process.