Network Pharmacology Analysis of Anticonvulsant Effect of Xiao Chai Hu Tang

Weikaixin Kong¹, Weiran Huang¹, Zhuo Huang¹,*, and Zhengwei Xie²,*

¹ Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Sciences, Peking University Health Science Center, 38 Xueyuan Lu, Haidian district, Beijing 100191, China
² Peking University International Cancer Institute and Department of Pharmacology, School of Basic Medical Sciences, Peking University Health Science Center, 38 Xueyuan Lu, Haidian district, Beijing 100191, China
*Corresponding author’s e-mail: xiezhengwei@hsc.pku.edu.cn; huangz@hsc.pku.edu.cn

Abstract. Epilepsy is the second largest disease after headache in the neurology department of the hospital. Previous studies have shown that Xiao Chai Hu Tang (XCHT) has a certain therapeutic effect on epilepsy. We used the gene expression array data of epilepsy patients, and the relevant data of the Chinese medicine components in the TCMSP database and DrugBank database, combined with bioinformatics analysis methods, to construct herbs-compounds-targets (HCT) network and protein-protein interaction (PPI) core network of XCHT. The results suggest that Caveolin 1 (CAV1) may be a key target in the treatment of epilepsy, and β-carotene may have potential antiepileptic activity. In addition, the PPI core function network is enriched in the process of transcription factor regulation, and the related protein is located on the nuclear chromosome and closely related to the immune process, which provides a reference for other researchers.

1. Introduction
Epilepsy is a chronic disease characterized by abnormal brain discharge¹, and medical therapy is still the most common method in China, which may cause side effects such as ataxia and headache resulting in the poor compliance in patients. Therefore, it’s of vital importance to develop a safe and effective drug. The treatment of epilepsy with traditional Chinese medicine has received more and more attention from the medical community due to its long history, rich experience, and advances of overall adjustment, positive curative effect and small side effects.

XCHT² is one combination of the most famous herbs recorded in Shanghanlun written by Zhongjing Zhang in Han dynasty. XCHT contains seven component herbs: Bupleurum hamiltonii N. P. Balakrishnan (Chaihu), Scutellaria baicalensis Georgi (Huangqin), Pinellia ternata Breit. (Banxia), Zingiber officinale Roscoe (Shengjiang), Panax ginseng C. A. Meyer (Renshen), Glycyrrhiza uralensis Fisch. (Gancao), Ziziphus jujuba Mill. (Dazao), operating the functions of maintaining the homeostasis of Shaoyang, promoting the metabolism of liver and gallbladder, and helping the transport of material in the body. XCHT has long been used in clinical practice for treating central nervous system diseases such as depression³, pain³, epilepsy³. However, at present, the pharmacological mechanism of XCHT against epilepsy is not yet clear, and the lack of relevant
bioinformatics analysis which provide guidance for the experiments is also a dilemma. So in this research, we integrated the genomics data of patients and network pharmacology analysis methods, and found the key pathways and targets of XCHT in the process of anticonvulsant.

2. Method

2.1. Data preparing and dealing
According to Matigian et al., we downloaded the gene array data of the twins containing the control samples and the absence epilepsy from the GEO database (GSE7486). In order to eliminate the interference of genetic factors, we used only twins in which both have epilepsy as the experimental group (8 cases), and twins in which both are free of epilepsy as the control group (12 cases). Wilcoxon test is applied to screen differentially expressed genes (DEGs), in which the filter criteria were \(|\log_{2}\text{FoldChange}|>0.5, p<0.05\). In order to extract the effective chemical ingredients in XCHT, TCMSP database was used to extract the active ingredients of the seven herbs. The extraction criteria were: bioavailability> 30\%, drug-likeness > 0.18. The target of the active ingredient is obtained from the TCMSP database and DrugBank database. Proteins that are both in DEGs and the targets of XCHT are regarded as the key targets of XCHT in the treatment of epilepsy, and the corresponding genes are the key genes. The dealing process was completed using the "limma" package in R (3.6.2), and the HCT interaction network was visualized using cytoscape software (3.6.1).

2.2. Bioinformatics analysis method
For the key genes found in the treatment of epilepsy with XCHT, we conducted a PPI network analysis. The database sources selected when extracting related genes of key genes include "DIP", "BIOGRID", "HPRD", "INTACT", "MINT" and "BIND".

After that, we extracted the core network in the obtained PPI network and screened it in two steps. First, the gene nodes with degree centrality less than 10 were removed; then, the gene nodes with betweenness centrality less than 350 were removed. The network formed by the left gene nodes can be regarded as the PPI core role network. The above process of constructing the PPI network and extracting the core network is completed through the "Bisogenet" and "CytoNCA" packages in Cytoscape. In the core network and epilepsy-related DEGs, we used the tags derived from the GO database and KEGG database to perform pathway enrichment analysis on the corresponding genes, and obtained enriched GO term and KEGG pathways, which provides a reference to understand the pharmacological process of XCHT in treating epilepsy.

3. Result

3.1. Result of differential genes
There were total of 399 up-regulated genes and 442 down-regulated genes in 8 epilepsy samples (Figure 1A, 1B), which indicated that changes in transcriptome levels can be more easily detected in epilepsy. Figure 1B shows the expression of the top 40 genes in the rank of |\log_{2}\text{FoldChange}|. Then enriched analysis of GO and KEGG was applied to DEGs. The related results are shown in Table 1. DEGs are closely related to the construction and function of organs. Related studies show that epilepsy often leads to multiple organ dysfunction, which is consistent with our results.

3.2. Medicinal HCT interaction network
The TCMSP database was employed to obtain the chemical composition of 7 herbs that can be effectively absorbed (bioavailability> 30\%, drug-likeness > 0.18): Ziziphus jujub Mill. (29), Zingiber officinale Roscoe(6), Scutellaria baicalensis Georgi(36), Glycyrrhiza uralensis Fisch. (92), Bupleurum hamiltonii N. P. Balakrishnan (17), Panax ginseng C. A. Meyer(22), Pinellia ternata (Thunb.) Breit. (13). At the same time, we obtained the potential molecular targets of these compounds provided in the TCMSP database and the DrugBank database. Then these targets and epilepsy-related DEGs were
intersected, and 11 key genes was obtained including CHRM3, CHRM4, GABRA1, PPARG, CA2, HMOX1, GSTM1, GSTM2, CAV1, IL1A, HSD3B2. The medicinal HCT interaction network based on 11 genes is shown in Figure 2. It can be seen that there are no more than three compounds interacting with HSD3B2, IL1A, CAV1, HMOX, GSTM1, and GSTM2 proteins, but there are more compounds that can bind to CA2, PPARG, CHRM3, CHRM4, and GABARA1 proteins. For compound molecules, MOL000098 (Quercetin) and MOL000422 (Kaempferol) can bind to more than three proteins and have stronger regulatory capabilities. For medicinal materials, Glycyrrhiza uralensis Fisch. (blue) has the most compounds in the network, which may be related to the abundant chemical components existing in the licorice that can be effectively absorbed.

Although there are no specific chemical components in Bupleurum hamiltonii that act on key genes, the four common compounds MOL000422, MOL000098, MOL000354, and MOL000449 all exist in Bupleurum.

![Figure 1. Differentially expressed genes. (A) Volcano map of DEGs. Red dots are up-regulated genes in epilepsy patients, green dots are down-regulated genes. (B) Heatmap of DEGs. Red bars are epilepsy patients, blue bars are control samples.](image)

![Table 1. Analysis of GO Enrichment in Differentially expressed genes](table)

| ID | Description          | Gen | BgRatio  | p     | geneID                                |
|----|----------------------|-----|----------|-------|---------------------------------------|
| GO:004 3583 | ear development | 14/2 | 11 212/17913 | 0.001 | BMP5/TIFAB/DLX5/SLC17A8/ECE1/W NT5A/OTX1/TWIST1/FREM2/CTHR1/NIK3-2/SIX4/FZD6/SALL1 |
| GO:004 2471 | ear morphogenesis | 10/2 | 11 115/17913 | 0.002 | TIFAB/DLX5/WNT5A/OTX1/TWIST1/CTHR1/NIK3-2/SIX4/FZD6/SALL1 |
| GO:009 0596 | sensory organ morphogenesis | 14/2 | 11 250/17913 | 0.002 | TIFAB/DLX5/WNT5A/CDON/OTX1/TWIST1/CTHR1/ROM1/NIK3-2/2/SIX4/FZD6/SALL1 |
3.3. PPI network and its core network

The PPI network constructed on the basis of 11 key genes, including 434 nodes and 4345 edges as shown in Figure 3A. After removing the proteins' regulatory effect on itself in the network, the proteins with degree centrality not less than 10 are highlighted (Figure 3B). In the degree centrality ranking (Table 2), CAV1 (degree=199) and PPARG (degree=153) ranked first and second respectively, which illustrates their key role in the regulatory pathway. The PPI network obtained after the removal of proteins with degree less than 10 has 261 nodes and 334 edges (Figure 3C). To further simplify the network, proteins with an betweenness centrality less than 350 are eliminated. As shown in Figure 3D, 44 nodes and 401 edges remain in the network. CAV1 (betweenness=7611.23) and PPARG...
(betweenness=3247.63) ranked first and fourth respectively, further verifying the important role of the two proteins.

Based on the remaining 44 genes, we performed GO and KEGG enrichment analysis. In the GO enrichment analysis (Figure 4A), 44 proteins are involved in the regulation process of transcription factors, which indicates that the core network of PPI is in the upstream stage of regulation process related to the occurrence and development of epilepsy. In addition, 44 proteins have also been enriched in many immune processes. At the cellular level, these proteins are more closely related to changes in the nucleus. At the molecular level, these proteins participate in the binding process of ubiquitin-like protein ligases, and are also closely related to changes in chromosome structure and function. In the KEGG enrichment analysis (Figure 4B), these proteins were enriched in the pathways of prostate cancer, viral infection, and body immunity, which indicates that the progression of epilepsy disease is closely related to the degree of collective immunity just like cancer9.

Figure 2. Medicinal HCT interaction network. The triangle is the key gene, and the rectangle is the chemical composition. In the chemical composition, blue comes from licorice, pink comes from ginger, red comes from jujube, green comes from pinellia, yellow comes from ginseng, and gray comes from a variety of medicinal herbs.

4. Discussion
In the procession of constructing medical HCT network, we can see that XCHT has the characteristics of simultaneous intervention of multiple targets for the treatment of epilepsy. In the PPI network,
CAV1 has the highest degree of centrality and betweenness centrality, which suggests that CAV1 may be an important target in epilepsy. Related studies have shown that CAV1 is involved in the formation and localization of plasma membrane microcapsules, and has the functions of mediating membrane vesicle transport, maintaining cellular cholesterol homeostasis, and regulating signal transduction. CAV1 is related to physiological or pathological changes of brain function, and plays an important role in neural development and synaptic plasticity. In the medical HCT network, both MOL000098 (Quercetin) and MOL002773 (β-carotene) can act on CAV1. There is sufficient evidence\textsuperscript{11} that quercetin has anticonvulsant and memory-enhancing effects, but research on the role of β-carotene in epilepsy is still very limited, and our work provides a reference for the next research. In addition, γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS)\textsuperscript{12}. It exerts an inhibitory effect through GABA receptors located in the brain, and the dysfunction of its receptor is associated with idiopathic epilepsy closely. When the level of GABA in the brain decreases and the level of excitatory neurotransmitters (such as glutamate) increases, epilepsy may occur. In the medical HCT network, flavonoids such as MOL000098 (Quercetin) and MOL000422 (Kaempferol) can effectively regulate the function of GABA receptors, and thus play an anticonvulsant effect, which also reflects the reasonableness and reliability of results. The compounds in our network which has not been reported in the literature may have antiepileptic activity.

Figure 3. PPI interaction network and its core network. The pink nodes are 11 core nodes. The nodes highlighted in yellow are those selected by Degree (B) or Betweenness (C).
Table 2. Analysis of GO Enrichment in DEGs

| Gene      | Degree | Gene      | Betweenness |
|-----------|--------|-----------|-------------|
| CAV1      | 199    | CAV1      | 7611.23     |
| PPARG     | 153    | EGFR      | 3460.04     |
| NTRK1     | 115    | NTRK1     | 3314.70     |
| ESR1      | 112    | PPARG     | 3247.63     |
| EGFR      | 111    | ESR1      | 2864.37     |
| APP       | 82     | APP       | 2163.92     |
| UBC       | 80     | UBC       | 1759.30     |
| HSP90AA1  | 79     | VCP       | 1551.53     |
| EP300     | 79     | XPO1      | 1434.17     |
| SRC       | 78     | EP300     | 1421.07     |

Figure 4. GO and KEGG enrichment analysis of related genes in PPI core network. (A) GO. BP, CC, and MF represent the enrichment results at the individual, cellular, and molecular levels, respectively. (B) KEGG.
5. Conclusion
We used the gene expression array data of epilepsy patients, and the relevant data of the Chinese medicine components in the TCMS database and DrugBank database, combined with bioinformatics analysis methods, to construct the HCT network and PPI core network of XCHT. The result suggests that CAV1 may be a key target in the treatment of epilepsy, and β-carotene may have potential antiepileptic activity. In addition, the PPI core function network is enriched in the process of transcription factor regulation, and the related protein is located on the nuclear chromosome and closely related to the immune process, which provides a reference for other researchers.

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