Prediction of the Anti-inflammatory Mechanism of Clematis chinensis based on Network Pharmacology

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Abstract. Clematis chinensis is a traditional Chinese medicine with good anti-inflammatory effects, but the underlying molecular mechanism is unclear. In this work, the potential mechanism of Clematis chinensis with anti-inflammatory effect was explored using the network pharmacology. The active chemical constituents of Clematis chinensis were screened according to the pharmacodynamic activities and ADME parameters, then the potential anti-inflammatory targets of the active chemical constituents in Clematis chinensis were screened with the databases of TCMSP, PubChem, TCMID, etc., and the target data sets were established through the BATMAN-TCM database, traditional Chinese medicine target database, HIT database, and TTD database. The targets related to inflammation were screened by using OMIM database, and the interactive target between clematis chinensis and inflammation were constructed by using PPI database. The complex network map of "drug composition-target-disease" was constructed by Cytoscape software, and GO (gene function) analysis was carried out by using ClueGO plug-in. Protein interaction analysis, target gene function enrichment analysis, and signal pathway analysis were carried out by STRING database, biological information annotation database (DAVID), and KEGG Pathway database. 39 out of 458 chemical constituents of Clematis chinensis had good drug-likeness and activity. 35 key targets were screened out by Degree, Betweenness Centrality, Closeness Centrality, and other network topology characteristics, and these targets were involved in 25 signaling pathways related to the anti-inflammatory action including TNF signaling pathway, MAPK signaling pathway, NF-kappa B signaling pathway, etc. In addition, it was also related to the positive and negative regulation of many biological processes such as inflammatory response, positive regulation of I-kappaB, kinase/NF-kappaB signaling, positive regulation of NF-kappaB, etc. The TNF signaling pathway, MAPK signaling pathway, and NF-kappa B signaling pathway were closely related to the anti-inflammatory activity of Clematis chinensis. Network pharmacology could be used to study the anti-inflammatory molecular mechanism of Clematis chinensis, which is beneficial to the development and utilization of Clematis chinensis.

1. Introduction

There are three sources of Clematis chinensis, which are *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr. The dried roots and rhizomes are used as Clematis chinensis herbs, and it has many traditional functions like dispelling rheumatism, dredging meridians, collaterals, etc. Clematis chinensis is mainly used to treatment of rheumatic arthralgia, limb numbness,
muscle and pulse contracture, flexion, and extension adverse [1]. Modern studies have shown that Clematis chinensis mainly contains saponins, flavonoids, lignans, phenols, alkaloids, and other components [2-6]. Interestingly, these components have anti-inflammatory, analgesic, anti-tumor, antibacterial, and other pharmacological activities [7-12]. At present, many studies have reported that Clematis chinensis has good anti-inflammatory activity, but the underlying mechanism is still unclear. However, the network pharmacology with holistic and systematic characteristics provide a new idea and method for revealing the anti-inflammatory mechanism of Clematis chinensis, which coincides with the "multi-component, multi-pathway and multi-target" synergetic characteristics of traditional Chinese medicine [13-15]. Therefore, in this paper, network pharmacology was used to analyse the possible targets and signalling pathways of the active chemical constituents for preventing and treating inflammation in Clematis chinensis, and the results could provide a reference for the further study of Clematis chinensis.

2. Method

2.1. The molecule structure and target protein prediction

The chemical components were obtained from the relevant literature (likely CNKI database, PubMed database), PubChem database, TCMSP database, etc. Then, the potential components were screened out using Pharmacological activity and ADME parameters. Based on the comprehensive analysis of BATMAN-TCM database and traditional Chinese medicine target database, Score cutoff (> 35 score) and P-value (p< 0.05) were used as indexes to screen the active constituents and predict the targets of Clematis chinensis. According to the similarity comparison, the active component database of Chinese herbal medicine (HIT, http://lifecenter.sgst.cn/hit/) and the control target database (TTD, http://bidd.nus.edu.sg/group/cjttd/) were used to screen the active constituents and its target data set was constructed. Human gene and gene phenotype database (OMIM, http://www.omim.org/) was used to screen the anti-inflammatory genes and protein targets of Clematis chinensis, and the data set of anti-inflammatory targets was established. Human target connexins were obtained through an interactive protein database (http://dip.doe-mbi.ucla.edu). Finally, all the selected targets were transformed into UniProt ID format by UniProt database query.

2.2. Network construction and topological profile analysis

Through PPI (http://www.genome.jp/kegg/) analysis, the active components, corresponding targets, inflammatory targets and interactive protein targets were connected into the "component-target-disease" network. The above network was visualized using Cytoscape 3.6.1 software. Among the topological parameters, degree and betweenness centrality were used as crucial factors to describe the most influential nodes in network. Thus, the node values higher than the median value of all nodes were chosen as the hubs.

2.3. Enrichment Analysis of Biological process and Pathway Diagram

The STRING database (https://string-db.org/) was used to analyze the protein-protein interaction of the screened targets. Finally, DAVID (https://david.ncifcrf.gov/) database) was used to analyze the KEGG pathway and the biological process of GO (Gene Ontology).

3. Result

3.1. Network construction

Through the literature, from PubChem database and TCMSP database, a total of 458 chemical constituents were obtained, of which 39 were the potential constituents. In addition, 685 interactive proteins were screened by Cytoscape software. The anti-inflammatory interactive network of Clematis chinensis was constructed by network pharmacology. After visualized with different colors and shapes, the network relationship between active components and disease targets could be directly seen in figure 1. Among them, the yellow square represented the common target for drugs and diseases, and it was
also the most important protein target of Clematis chinensis for its anti-inflammatory activity. The yellow ellipse represented the direct target of inflammation, the red triangle represented the predicted active compound in Clematis chinensis, the blue ellipse represented the direct target of active components of Clematis chinensis, and the purple ellipse represented the interacting protein banded to the components of Clematis chinensis to the disease targets.

Fig. 1 "Component-Target-Disease" Interactive Network for Anti-inflammatory Action of the active components in Clematis chinensis

3.2. Topological profile analysis
The protein targets associated with the active components of Clematis chinensis were obtained, and the topological parameters of these protein targets were calculated. The median value of the three topological parameters (Degree, Betweenness centrality and Closeness centrality) was 6, 0.005, and 0.173, respectively. The selected targets with three topology parameters of each target greater than the median values were selected as the important target proteins in the anti-inflammatory activity of Clematis chinensis. Finally, 35 targets were screened out, and the results were shown in Table 1. The protein interaction of the selected targets were shown in Fig. 2. The protein interaction showed that the active components of Clematis chinensis are interaction with each other and regulation each other.

Table. 1 Topological parameters related to the Target of Anti-inflammatory effects of active components of Clematis chinensis

| Uniprot ID | Protein names              | Gene names | Closeness Centrality | Degree | Betweenness Centrality |
|-----------|---------------------------|------------|----------------------|--------|------------------------|
| P05067    | Amyloid-beta A4 protein   | APP        | 0.193                | 12     | 0.033                  |
| P31749    | RAC-alpha serine/threonine-protein kinase | AKT1 | 0.234                | 13     | 0.029                  |
| P41182    | B-cell lymphoma 6 protein | BCL6       | 0.217                | 8      | 0.019                  |
| Q13936    | Voltage-dependent L-type calcium channel subunit alpha-1C | CACNA1C | 0.176                | 8      | 0.019                  |
| Q14790    | Caspase-8                 | CASP8      | 0.193                | 6      | 0.009                  |
| P24385    | G1/S-specific cyclin-D1   | CCND1      | 0.247                | 10     | 0.041                  |
| P00533    | Epidermal growth factor receptor | EGFR | 0.220                | 19     | 0.040                  |
| Q13158    | FAS-associated death domain protein | FADD | 0.189                | 7      | 0.011                  |
| P04150    | Glucocorticoid receptor   | NR3C1      | 0.237                | 17     | 0.035                  |
| Q16665    | Hypoxia-inducible factor 1-alpha | HIF1A | 0.191                | 12     | 0.025                  |
| P25963    | NF-kappa-B inhibitor alpha | NFKBIA     | 0.219                | 18     | 0.036                  |
| Gene ID | Name                                      | Transcript | Score | p-value |
|--------|-------------------------------------------|------------|-------|---------|
| O15111 | Inhibitor of nuclear factor kappa-B kinase subunit alpha CHUK | 0.211      | 18    | 0.021   |
| O14920 | Inhibitor of nuclear factor kappa-B kinase subunit beta IKBKB | 0.225      | 15    | 0.018   |
| P05231 | Interleukin-6 IL6 | 0.186      | 6     | 0.014   |
| P05412 | Transcription factor AP-1 JUN | 0.237      | 15    | 0.056   |
| Q99558 | Mitogen-activated protein kinase kinase kinase 14 MAP3K14 | 0.209      | 13    | 0.020   |
| Q99759 | Mitogen-activated protein kinase kinase kinase 3 MAP3K3 | 0.200      | 10    | 0.022   |
| Q9Y6K9 | NF-kappa-B essential modulator IKBKG | 0.226      | 23    | 0.046   |
| P19838 | Nuclear factor NF-kappa-B p105 subunit NFKB1 | 0.191      | 20    | 0.017   |
| P35228 | Nitric oxide synthase, inducible NOS2 | 0.265      | 12    | 0.070   |
| P04637 | Cellular tumor antigen p53 TP53 | 0.263      | 60    | 0.201   |
| O15350 | Tumor protein p73 TP73 | 0.202      | 9     | 0.006   |
| P09874 | Poly [ADP-ribose] polymerase 1 PARP1 | 0.212      | 11    | 0.031   |
| P23219 | Prostaglandin G/H synthase 1 PTGS1 | 0.237      | 14    | 0.010   |
| P35354 | Prostaglandin G/H synthase 2 PTGS2 | 0.241      | 18    | 0.025   |
| P37231 | Peroxisome proliferator-activated receptor gamma PPARG | 0.240      | 17    | 0.029   |
| Q13546 | Receptor-interacting serine/threonine-protein kinase 1 RIPK1 | 0.228      | 7     | 0.064   |
| Q13586 | Stromal interaction molecule 1 STIM1 | 0.187      | 7     | 0.011   |
| Q86WV6 | Stimulator of interferon genes protein TMEM173 | 0.213      | 8     | 0.010   |
| Q04206 | Transcription factor p65 RELA | 0.195      | 19    | 0.022   |
| P02786 | Transferrin receptor protein 1 TFRC | 0.174      | 7     | 0.008   |
| P01375 | Tumor necrosis factor TNF | 0.215      | 10    | 0.158   |
| P19438 | Tumor necrosis factor receptor superfamily member 1A TNFRSF1A | 0.202      | 10    | 0.030   |
| P25445 | Tumor necrosis factor receptor superfamily member 6 FAS | 0.217      | 11    | 0.096   |
| Q9Y4K3 | TNF receptor-associated factor 6 TRAF6 | 0.215      | 22    | 0.044   |
3.3. Biological function analysis of GO

25 potential targets were mapped to the DAVID database for bio-functional enrichment and systematic analysis of their biological processes. 296 biological processes were enriched, 20 of which are $P \leq 0.01$ and Count $\geq 10$, and the results were shown in Table 2. The results showed that these targets are involved in a variety of biological processes including inflammatory response, positive regulation of I-kappaB, kinase/NF-kappaB signaling, positive regulation of NF-kappaB, etc. These biological processes were closely related to the occurrence and development of inflammation. It indicates that the pathogenesis of inflammation is involved in the abnormality of many biological processes in vivo, and these screened biological processes might be related to the anti-inflammatory effects of Clematis chinensis.

**Table 2. GO of bioaccumulation Analysis of Anti-inflammatory effect of Clematis chinensis**

| Category                  | Term                                           | Count | Count (%) | P-Value   |
|---------------------------|------------------------------------------------|-------|-----------|-----------|
| GOTERM_BP_DIRECT          | positive regulation of transcription from RNA polymerase II promoter | 23    | 65.7      | 1.6E-19   |
| GOTERM_BP_DIRECT          | inflammatory response                           | 13    | 37.1      | 5.2E-12   |
| GOTERM_BP_DIRECT          | positive regulation of I-kappaB kinase/NF-kappaB signaling | 12    | 34.3      | 1.1E-14   |
| GOTERM_BP_DIRECT          | positive regulation of apoptotic process         | 11    | 31.4      | 2.6E-10   |
| GOTERM_BP_DIRECT          | apoptotic process                                | 11    | 31.4      | 1.1E-07   |
| GOTERM_BP_DIRECT          | positive regulation of NF-kappaB transcription factor activity | 10    | 28.6      | 4.1E-12   |
| GOTERM_BP_DIRECT          | negative regulation of apoptotic process         | 10    | 28.6      | 2.1E-07   |
| GOTERM_BP_DIRECT          | positive regulation of transcription, DNA-templated | 10    | 28.6      | 5.9E-07   |
| GOTERM_BP_DIRECT          | negative regulation of transcription from RNA polymerase II promoter | 10    | 28.6      | 9.3E-06   |
3.4. **KEGG pathway analysis**

The results showed that 25 signaling pathways (P≤0.01 and Count≥10) related to the anti-inflammatory effect of Clematis chinensis were screened out by KEGG pathway enrichment analysis (Table 3). These pathways have shown a direct or indirect role in anti-inflammatory, such as TNF signaling pathway, MAPK signaling pathway, and NF-kappa B signaling pathway.

| Category          | Term                                           | Count | Count (%) | P-Value |
|-------------------|------------------------------------------------|-------|-----------|---------|
| KEGG_PATHWAY      | Pathways in cancer                            | 20    | 57.1      | 1.4E-15 |
| KEGG_PATHWAY      | TNF signaling pathway                         | 17    | 48.6      | 6.4E-21 |
| KEGG_PATHWAY      | Chagas disease (American trypanosomiasis)     | 16    | 45.7      | 2.5E-19 |
| KEGG_PATHWAY      | MAPK signaling pathway                        | 16    | 45.7      | 2E-13   |
| KEGG_PATHWAY      | Apoptosis                                      | 15    | 42.9      | 5.8E-21 |
| KEGG_PATHWAY      | Hepatitis B                                    | 15    | 42.9      | 1.7E-15 |
| KEGG_PATHWAY      | Herpes simplex infection                      | 15    | 42.9      | 4.7E-14 |
| KEGG_PATHWAY      | HTLV-1 infection                               | 15    | 42.9      | 4.4E-12 |
| KEGG_PATHWAY      | Toll-like receptor signaling pathway           | 14    | 40        | 9E-16   |
| KEGG_PATHWAY      | Osteoclast differentiation                     | 13    | 37.1      | 5.3E-13 |
4. Discussions
In this paper, the network pharmacology methods were used to predict the anti-inflammatory targets and pathways of the active chemical constituents from Clematis chinensis, and 39 active chemical constituents were screened out. The interactive network diagram of "component-target-disease" was constructed. The results showed that 25 pathways and 20 biological processes are directly or indirectly involved in the anti-inflammatory effects of Clematis chinensis through pathway enrichment analysis, and the main signaling pathways included TNF signaling pathway, MAPK signaling pathway, NF-kappaB signaling pathway and the biological processes were inflammatory response, positive regulation of I-kappaB, kinase/NF-kappaB signaling, positive regulation of NF-kappaB, and so on. Finally, the research for anti-inflammatory mechanism of Clematis chinensis biological effects with multi-component, multi-target, and multi-pathway were achieved. The predicted results suggest that there are three potential anti-inflammatory signaling pathways involved in the anti-inflammatory effects of Clematis chinensis, and the next step is to select the NF-kappaB signaling pathway, which is most closely related to the pathogenesis of inflammation, to be verified. The aim of this work is to provide the reference for the in-depth study of Clematis chinensis.

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References

[1] National Pharmacopoeia Commission. Pharmacopoeia of the people's Republic of China. A [M]. Beijing: China Medical Science and Technology Press, 2015: 250-251

[2] Shi S, Jiang D, Dong C, et al. Triterpene saponins from clematis mandshurica.[J]. Journal of Natural Products, 2006, 69(11):1591-1595.

[3] Gong Y X, Hua H M, Xu Y N, et al. Triterpene Saponins from Clematis mandshurica and Their Antiproliferative Activity[J]. Planta Medica, 2013, 79(11):987-994.

[4] Dong C X, Shi S P, Wu K S, et al. Studies on chemical constituents from root of Clematis hexapetala[J]. China Journal of Chinese Materia Medica, 2006, 31(20):1696.

[5] Zhang W K, Yang G E, Li Q, et al. Studies on chemical constituents of Euphorbia sororia.[J]. China Journal of Chinese Materia Medica, 2006, 31(20):1694.

[6] Liu J Y, Zhou N, Gong Y X, et al. Isolation and identification of chemical constituents from Clematis mandshurica[J]. Chinese Journal of Medicinal Chemistry, 2016,26(01):56-60.

[7] Fu Q, Zan K, Zhao M, et al. Triterpene saponins from Clematis chinensis and their potential anti-inflammatory activity.[J]. Journal of Natural Products, 2010, 73(7):1234.

[8] Liu Y., Shi R.b., Liu B., etc. Study on HPLC fingerprint of Chemical constituents of Salvia miltiorrhiza Bunge [J]. Journal of Beijing University of traditional Chinese Medicine, 2006, 29 (3): 188-192.

[9] Zhao Ying, Yu Chunfan, Zhang Guiying, et al. Antitumor effect of total saponins of Clematis chinensis and its effect on proliferation cycle of cancer cells [J]. Shizhen Medical Chinese Medicine, 2010, 21 (08): 1908-1909.

[10] Yun-Yi Z, Hong-Wei Z, Pei-Feng L I, et al. Antispasmodic Anti-inflammation and Analgesic Effect of Clematis[J]. Chinese Traditional Patent Medicine, 2001 11: 30-33.

[11] Xin-Rong Z, Chang-Jiang W, Xiao-Qin W. Effect of Extracts from Clematidis Chinensis Radix et Rhizoma on Diabetic Nephropathy in Rats[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2015,21(16):152-156.

[12] Chen F Y, Xiang Jie G U, Zhong M K. Effect of sixpetal clematis root parenteral solution on interlukin-1 level in osteoarthritis joint fluid and chondrocyte culture supernatant[J]. Orthopedic Journal of China, 2004(07):43-45.

[13] Hartog A, Hougee S, Faber J, et al. The multicomponent phytopharmaceutical SK1306X inhibits in vitro cartilage degradation and the production of inflammatory mediators[J]. Phytotherapy Research & Phytopharmacology, 2008, 15(5):313-320.

[14] Yan D, Xiao X. [Investigation on pattern of quality control for Chinese materia medica based on famous-region drug and bioassay--the work reference].[J]. China Journal of Chinese Materia Medica, 2011, 36(9):1249-1252.

[15] Liang X, Li H, Li S. A novel network pharmacology approach to analyse traditional herbal formulae: the Liu-Wei-Di-Huang pill as a case study[J]. Molecular Biosystems, 2014, 10(5):1014-1022.