Original Article

Utilizing network pharmacology to explore the underlying mechanism of wenshenxuanbi decoction in treating osteoarthritis

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Abstract: Objective: The network pharmacology method was adopted in this study to establish the relationship between “Efficacy of ingredients-disease targets-biological pathway” and screening the targets of the wenshenxuanbi decoction of the treatments for osteoarthritis and clarifying its mechanism of treatment. Methods: Chemical components and selected targets related to eleven herbs were searched in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. In the GeneCards database, the OMIM database, and the DisGeNET database, osteoarthritis disease targets were searched. Then we screened the core targets between the drugs and the disease by identifying the intersections. Then an interaction network diagram of the targets was constructed using String. The GO Enrichment and KEGG enrichment analyses of the targets were analyzed based on the DAVID database. Results: Selecting oral bioavailability ≥ 30% and drug likeness ≥ 0.18 as search filters, 181 active ingredients and 134 corresponding protein targets were screened for the wenshenxuanbi decoction. There were 72 core genes that intersected with 2171 disease-related genes in OA. The GO analysis contained a total of 304 enrichment results, including 211 biological processes, 42 cell compositions, and 51 molecular functions. A total of 73 pathways were enriched by KEGG. This study predicted the main possible mechanisms of wenshenxuanbi decoction in treating OA, including anti-inflammation, regulating cellular proliferation, differentiation and apoptosis, promoting the balance between osteogenesis and osteoclasts, and antioxidation. Keywords: Network pharmacology, Wenshenxuanbi decoction, osteoarthritis, target

Introduction

Osteoarthritis (OA) is a common chronic degenerative disease in adults [1, 2]. There are no effective drugs in modern medicine for treating OA [3-7]. Moreover, 10% of the global medical behaviors are related to OA [3]. OA is often referred to as “arthraigia” in traditional Chinese medicine [8]. Traditional Chinese medicine has certain advantages in treating OA. Wenshenxuanbi decoction has a good clinical effect on OA [9]. Wenshenxuanbi decoction consists of monkshood 10 g, cassia twig 10 g, Woodwardia 10 g, asarum 6 g, poria cocos 12 g, coix seed 15 g, banksia rose 10 g, gastrodia elata 10 g, dogwood 10 g, alisma 10 g, stir-baked rhizoma atractyloides macrocephalae in bran 10 g, and licorice 10 g. However, the mechanism by which wenshenxuanbi decoction treats OA is unclear.

Nowadays, network pharmacology is widely used to explore the pharmacological mechanisms of traditional Chinese medicine, treating disease [10-12]. We utilized network pharmacology to explore the potential mechanism of wenshenxuanbi decoction in treating OA.

Methods

Screening the active compounds in wenshenxuanbi decoction and gathering potential targets

This study is based on the traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php), a pharmacology database and analysis platform of the TCM system, which retrieves all the chemical components of the twelve traditional Chinese medicines in the wenshenxuanbi decoction with keywords. In this study, oral availability (OB) ≥ 30% and drug likeness (DL) ≥ 0.18 were used as the screening conditions for the active compounds. In combination with published studies, the chemi-
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Identification of disease targets

The OA-associated disease targets were searched in GeneCards (https://www.genecards.org/), OMIM (http://www.omim.org/), and DisGeNET (http://www.disgenet.org/web/DisGeNET/menu/home), three disease-related databases, with “osteoarthritis” as a keyword.

Construction of the network

The relevant action targets of wenshenxuanbi decoction and the known disease targets of OA were intersected, and the intersection targets were uploaded to the STRING database (http://string-db.org/) [13] for building the protein-protein interaction (PPI) network.

Construction of the compound-target-disease network and screening the key genes

The selected core targets were uploaded to Cytoscape 3.2.1 software (https://cytoscape.org/) to generate a chemcompound-target-disease network graph to demonstrate the effect of the compound on the disease at the system level. At the same time, Cytoscape 3.2.1 software was used for the core network screening based on the topological parameters.

Enrichment analysis of the gene ontology (GO) function and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

To illustrate the role of the target of the Chinese medicine compound protein in the gene function, the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/, v.6.8) was used for the GO enrichment analysis [14]. The pathway enrichment analysis was accomplished using the Kyoto Encyclopedia of Genes and Genomes data (http://www.kegg.jp/) obtained from DAVID [15]. P < 0.05 was set as the basic screening condition for the GO enrichment analysis and the KEGG pathway enrichment analysis. The results of the enrichment analysis were then visualized through the OmicShare platform (http://www.omicshare.com/tools/home/report/koenrich.html), in order to reflect the main effect pathway of the wenshenxuanbi decoction for the treatment of osteoarthritis.

Results

Screening of the active compounds and targets

The compounds in the wenshenxuanbi decoction were collected using TCMSP, with OB ≥ 30% and DL ≥ 0.18 as the screening conditions. After we removed the duplicates, a total of 181 compounds were obtained, and they were considered as candidate compounds. In addition, 134 corresponding targets of the candidate compounds were obtained after we collected them and removed the duplicates on the TCMSP platform.

Screening of the core genes and the construction and analysis of the PPI graph network

A total of 2,307 targets were obtained by searching the Genecards, OMIM, and DisGeNET...
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Figure 2. The core targets protein-protein interaction network. MMP2, matrix metalloproteinase 2; NOS2, nitric oxide synthase 2; COL1A1, collagen, type I, alpha 1; PPP3CA, protein phosphatase 3-catalytic subunit-alpha isoform (calcineurin A alpha); CDK1, cyclin-dependent kinase-1; BCL2, B-cell CLL/lymphoma 2; F7, coagulation factor VII; HTR3A, 5-hydroxytryptamine receptor 3A; GSTP1, glutathione S-transferase pi-1; AHR, aryl hydrocarbon receptor; EGF, epidermal growth factor; PTGS2, prostaglandin-endoperoxide synthase 2; ESR1, estrogen receptor alpha gene; CDK4, cyclin-dependent kinase 4; POR, P450 (cytochrome) oxidoreductase; VEGFA, vascular endothelial growth factor A; CCNA2, cyclin A2; GSK3B, glycogen synthase kinase 3 beta; MMP1, matrix metalloproteinase 1; TRPV1, transient receptor potential cation channel, subfamily V, member 1; HMOX1, heme oxygenase 1; MMP3, matrix metalloproteinase 3; GSTM1, glutathione S-transferase M1; MAPK1, mitogen-activated protein kinase 1; PLAT, plasminogen activator tissue; EGFR, epidermal growth factor receptor; SOD1, superoxide dismutase 1; G6PD, glucose-6-phosphate dehydrogenase; MAPK8, mitogen-activated protein kinase 8; CDK2, cyclin-dependent kinase 2; PGR, progesterone receptor; ODC1, ornithine decarboxylase 1; CAT, catalase; IL1B, interleukin 1, beta; CALM1, calmodulin 1; ESR2, estrogen receptor 2; PTGER3, prostaglandin E receptor 3; PLAU, plasminogen activator, urokinase; SLC6A4, solute carrier family 6, member 4; ALOX5, arachidonate 5-lipoxygenase; GJA1, gap junction protein, alpha 1, 43 kDa;
DPP4, dipeptidylpeptidase 4; PON1, paraoxonase 1; TNFSF15, tumor necrosis factor (ligand) superfamily, member 15; PPARG, peroxisome proliferative activated receptor, gamma; SELE, selectin E; THBD, thrombomodulin; NR3C1, nuclear receptor subfamily 3, group C, member 1; MAPK14, mitogen-activated protein kinase 14; MPO, myeloperoxidase; ADRB2, adrenergic, beta-2, receptor, surface; CTSD, cathepsin D; CCL2, chemokine (C-C motif) ligand 2; IL6, Interleukin-6; HSP90AA1, heat shock protein 90 kDa alpha , class A member 1; COL3A1, collagen, type III, alpha 1; KDR, kinase insert domain receptor; IL2, Interleukin-2; HSPA5, heat shock 70 kDa protein 5; OPRD1, opioid receptor, delta 1; CA2, carbonic anhydrase II; OPRM1, opioid receptor, mu 1; TLR4, toll-like receptor 4; VCAM1, vascular cell adhesion molecule 1; IFNGR1, interferon gamma receptor 1; PTGS1, prostaglandin-endoperoxide synthase 1; F3, coagulation factor III; IFNB1, interferon, beta 1; JUN, v-jun sarcoma virus 17 oncogene homolog; AR, androgen receptor; CYP3A4, cytochrome -family 3-subfamily 4; TP63, tumor protein p63.

Figure 3. The compound-target-diseases of WSXBD.
removed) and 770 sides (the network nodes represent the targets, and the lines represent the interactions of the targets). The average node degree was 21.4.

**The compound-target-disease network**

Cytoscape3.2.1 software was used to construct a chemical-target-disease network graph (Figure 3) of 72 targets, 181 compounds, WSXBD and OA. With an average of 7.2 degrees of freedom, 18 core targets with greater than average degrees of freedom were selected using the core network based on the topological parameters. The top 5 targets were DPP4, AR, PTGS2, PPARG, ESR1, and NR3C1. Therefore, the combined action mechanism of Wenshenxuanbi decoction with multiple components and multiple targets in the treatment of osteoarthritis reflects the characteristics of the traditional Chinese medicine compound.

**Enrichment analyses of the GO function and KEGG pathway**

A total of 211 biological process (BP) enrichment results, 42 cell composition (CC) enrichment results, and 51 molecular function (MF) enrichment results were obtained. The top 20 GO enrichment results are shown in Figures 4-6. In the BP correlation analysis results, the top 20 enrichment results are positive regulation of the nitric oxide biosynthesis process, response to hypoxia, response to the drug,
response to ethanol, organ regeneration, negative regulation of the apoptotic process, response to toxic substances, positive regulation of the transcription from the RNA polymerase II promoter, positive regulation of the transcription-DNA-template, signal transduction, response to estrogen, positive regulation of cell proliferation, cellular response to mechanical stimuli, circadian rhythm, positive regulation of cell migration, positive regulation of gene expression, response to estradiol, response to hydrogen peroxide, regulation of sequence-specific DNA binding transcription factor activity, and angiogenesis (Figure 4). In the analysis results related to CC, the enrichment results of the top 20 are extracellular space, extracellular region, membrane raft, extracellular matrix, cytosol, plasma membrane, mitochondrion, cell surface, perinuclear region of cytoplasm, secretory granule, lysosome, extracellular exosome, focal adhesion, vesicle, Golgi apparatus, nucleus, caveola, dendrite cytoplasm, myelin sheath, and postsynapse (Figure 5).

Figure 5. A cellular component analysis of the GO pathway enrichment analysis for the active ingredient-potential targets of wenshenxuanbi decoction.

A total of 73 KEGG pathway enrichment results were obtained. The top 20 GO enrichment results are as follows (Figure 7). Pathways in cancer, Chagas disease (American trypanosomiasis), TNF signaling pathways, Tuberculosis, HIF-1 signaling pathway, Pertussis, PI3K-Akt signaling pathway, Salmonella infection, Bladder cancer, Estrogen signaling pathway, Leishmaniasis, influenza A, Hepatitis B, NOD-like receptor signaling pathway, Progesterone-mediated oocyte maturation, Rheumatoid arthritis, Prostate cancer, Focal adhesion, Osteoclast differentiation, Measles. This suggests that the treatment of osteoarthritis with wenshenxuanbi decoction may be regulated by the above signaling pathways.

Discussion

Considering the common signaling pathways of OA and excluding the pathways of unrelated diseases, we found that wenshenxuanbi decoction can effectively treat OA through its anti-inflammatory properties, affect proliferation, differentiation and apoptosis, and promote the balance between osteogenesis (OB) and osteoclasts (OC) and antioxidant.
(1) Anti-inflammatory. The enrichment analysis showed that the inflammation-related signaling pathways were enriched in the TNF related signaling and nod-like receptor pathways. Inflammatory factors [16, 17] exacerbate the inflammation, increase the protease expression of MMP-1, MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5, accelerate the degradation of cartilage by activating NF-κB, extracellular protein kinase (Erk)-c-Jun amino-terminal kinase (Jnk)-protein kinase p38 lightning (p38), mitogen activated protein kinase (MAPK), and the TNF signaling pathways [18-22]. β-sitosterol, the active components of cornus, inhibits the activity of IL-6 and reduces the secretions of IL-1, TNF-α by reducing the synthesis of NO [23, 24]. Quercetin, the active component of licorice root., plays a protective role on cartilage by inhibiting the NF-κB and TNF signaling pathways [25, 26]. Our previous study found that cinnamaldehyde, the active component of cassia twig, protects articular cartilage by inhibiting inflammation through the NF-κB and p38-JNK signaling pathways [20]. Proanthocyanidin, the active component of cassia twig, also decreases the expression of MMP-3 and MMP-13 by inhibiting the p38MAPK signaling pathway to protect cartilage. When hypoxia occurs in joints, HIF-1α will be transferred into the nuclei. It increases the expression of VEGF and angiogenesis, leading to synovitis [27, 28]. Our KEGG enrichment analysis suggests that wenshenxuanbi decoction plays an important role in treating OA by affecting HIF-1 signaling path-

Figure 6. A molecular function analysis of the GO pathway enrichment analysis for the active ingredient-potential targets of wenshenxuanbi decoction.
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way. (2) It affects the proliferation, differentiation, and apoptosis of cells, and promotes the balance between OB and OC. According to the enrichment results, the PI3K/Akt signaling pathway, the osteoclast differentiation related pathway, the estrogen signaling pathway, and the downstream pathways of the cancer signaling pathway, such as Wnt [29] and the MAPK signaling pathway [30], play important roles in treating OA. The PI3K/Akt signaling pathway is related to the estrogen signaling pathway [31], which is not only an important pathway for regulating chondrocyte apoptosis and growth [32], it also participates in subchondral bone remodeling by regulating the proliferation, differentiation, and apoptosis of osteoblasts and osteoclasts [33-37]. Quercetin promotes osteogenic differentiation of rat bone marrow mesenchymal stem cells (BMSCs) [25, 26]. Low concentrations of cinnamaldehyde significantly improve the activity of alkaline phosphatase in osteoblasts, and promote the proliferation, differentiation, and osteoblast functions of osteoblasts [38]. Cinnamic acid blocks the G0/G1 phase of rat BMSCs and promotes their differentiation into osteoblasts [39, 40]. (3) Antioxidant. The mitochondrial dysfunction of chondrocytes and synovial cells produces NO and leads to oxidative damage in cartilage. The active component of dogwood, β-sitosterol, increases the activity of superoxide dismutase and glutathione peroxidase and decreases the activity of catalase. Cinnamaldehyde inhibits the oxidation of chondrocytes by increasing the activity of antioxidant enzymes [25]. Cinnamomum polyphenols, the active component of cassia twig, also has a strong reducibility [41].

Conclusion

The main mechanisms of wenshenxuanbi decoction in treating OA are its anti-inflammatory properties, its ability to regulate cellular proliferation, differentiation, and apoptosis, and its ability to promote the balance between OB and OC and antioxidation.

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Disclosure of conflict of interest

None.

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