

Exploration on the Mechanism of Cryptotanshinone in Treating Kashin-beck Disease Based on Network Pharmacology

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Abstract: In order to explore the possible mechanism of cryptotanshinone (CTS) in the treatment of Kashin-beck disease (KBD) based on network pharmacology, we obtained CTS drug targets from TCMSp, CTD and DGIdb databases, and we searched Genecard, TTD, OMIM and Drugbank databases with "KBD" as the keyword to obtain the disease target of KBD. Drug-disease gene interaction was performed in Venny2.1.0 to obtain the intersection target. String database and Cytoscape 3.7.1 were used for protein-protein interaction network (PPI) analysis, and DAVID database was used for GO enrichment and KEGG enrichment pathway analysis. The "drug-target-pathway-disease" network diagram was constructed by Cytoscape. Resultly, a total of 10 key targets were selected, 125 items in 3 directions were obtained by GO enrichment analysis, and 34 signaling pathways were obtained by KEGG enrichment analysis. In conclusion, through network pharmacological analysis, CTS can treat KBD through multi-target and multi-pathway.

1. Introduction

Kaschin-beck disease is a kind of chronic and endemic orthopedic disease, and its early injury is mainly reflected in cartilage injury [1]. Deep necrosis of epiphysis, epiphysis plates, and articular cartilage occurs during childhood and adolescence. Clinical manifestations usually include joint pain, enlargement, bone growth retardation or deformity [2]. The disability rate of KBD is very high, and the living standards of the patients have been greatly reduced, which brings great burden to patients and their families [3]. In the course of treatment of Kashin-beck disease, there is still no specific drug that can alleviate the disease progression. Therefore, it is crucial to explore effective prevention and treatment of Kashin-beck disease drugs, improve the affected joints and improve the quality of life of patients. Screening from natural plants may be a good choice to improve the pain of KBD and ease the progression of the disease.

Salvia miltiorrhiza is a natural herbal medicine commonly used in clinic in China. It is used for a variety of orthopedic diseases, including fractures, arthritis and osteoporosis [4-6]. Studies have found

that salvia miltiorrhiza can inhibit oxidative damage, inflammatory mediators, protect OA cartilage, reduce pain, and ease the development of OA disease [7]. CTS is a fat-soluble active component of salvia miltiorrhiza. Experiments have shown that CTS can inhibit IL-1 β induced chondrocyte inflammation, effectively inhibit OA cartilage destruction, relieve OA pain, and delay the progression of OA disease [8]. In order to explore whether CTS can also inhibit cartilage destruction and relieve pain in KBD, and predict which targets and pathways through which CTS can treat KBD, combined with the rapidly developing and mature network pharmacology in recent years [9], explore the main targets and pathways involved in the treatment of KBD by CTS, and provide reference for related experiments and treatments.

2. Manuscript Preparation

2.1. Collection of cryptotanshinone targets

We searched for "Cryptotanshinone" in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <https://tcmisp.com/>) and Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>) and The Drug Gene Interaction Database Database (DGIdb, <https://www.dgldb.org/>). And we exported the results, and obtained the CTS target after summary and de-duplication. The CTS targets were processed through the Uniprot database (<https://www.uniprot.org/>), the target protein names were converted from the gene names, and the final key drug targets were obtained.

2.2. KBD disease target collection

We searched "Kashin beek-diseases" for KBD disease targets in Genecard database (<https://www.genecards.org/>), Drugbank database (<https://go.drugbank.com/>), OMIM database (<https://omim.org/>), disgenet Database (<https://www.disgenet.org/>). The above four targets was sorted and gained by the database to heavy, after entering the standard gene name in the Uniprot database.

2.3. Screening of common targets of drugs and diseases

After drawing tools, we imported targets of KBD and targets of CTS into venny2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html/>), and then we built Wayne figure, CTS and KBD intersection targets.

2.4. Construction of PPI diagram

The PPI was constructed in the String database (<https://www.string-db.org/>). The "Multiple Protein" option was selected, the intersection target was entered, the Protein attribute was selected as "Homo Protein", and the lowest interaction threshold was selected as Medium Confidence (0.400) for integration and evaluation. The result is imported into Cytoscape3.7.1 software to obtain a PPI visual network diagram.

2.5. Bioprocess enrichment and pathway enrichment analysis of the effects of CTS on KBD targets

We imported intersection targets into DAVID database (<https://david.ncifcrf.gov/>), selected the "OFFICIAL GENE SYMBOL", species selection for "Homo sapiens". The analysis Tool was selected as "Functional Annotation Tool", and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Screening by $P < 0.05$, and in the online mapping

software microscopic letter (<http://www.bioinformatics.com.cn/>), we drew up the corresponding graph.

3. Results

3.1. CTS drug targets and KBD disease targets

The chemical structure of CTS is shown in Figure 1. CTS were retrieved through TCMSP, CTD and DGIdb databases, and the results were sorted and de-duplicated into Uniprot database for target-gene matching. A total of 67 targets were obtained. KBD disease prediction targets were retrieved from GeneCard, Disgenet, OMIM and Dragbank databases, and 707 potential targets were obtained after integration and de-duplication. VENN2.1, an online mapping tool, was used to interact drug prediction targets and potential disease targets, and 10 intersecting targets (see Table 1) and Wayne diagram (see Figure 2) were obtained.

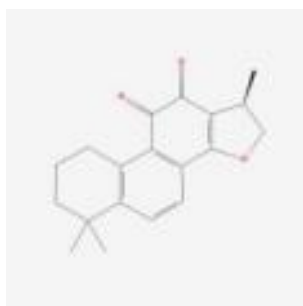


Figure 1: Chemical structure diagram of CTS

Table 1: Intersection table of CTS and KBD target genes

No.	Protein names	Gene names	Uniprot ID
1	Tumor necrosis factor	TNF	P01375
2	Caspase-3	CASP3	P42574
3	Catenin beta-1	CTNNB1	P35222
4	Glycogen synthase kinase-3 beta	GSK3B	P49841
5	Amyloid-beta precursor protein	APP	P05067
6	Mothers against decapentaplegic homolog 3	SMAD3	P84022
7	Prostaglandin G/H synthase 2	PTGS2	Q05769
8	Prostaglandin G/H synthase 1	PTGS1	P22437
9	Epidermal growth factor receptor	EGFR	P00533
10	Sequestosome-1	SQSTM1	Q13501

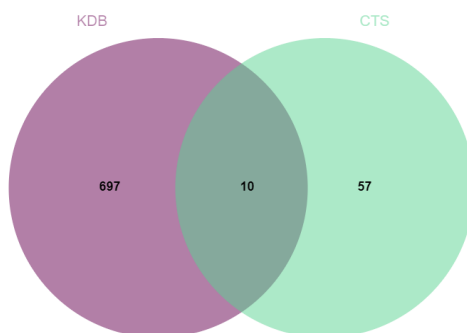


Figure 2: Venn intersection diagram of target proteins of KBD-CTS

3.2. PPI network analysis

The intersection target was imported into the String database, and the biological selection was Homo sapiens. Importing the result from the string database into cyto- scape3.7.1 gives a network diagram with 10 nodes and 37 edges. Node size and color are positively correlated with Degree value. Line thickness and color are positively correlated with the Combined score value, as shown in Figure 3.

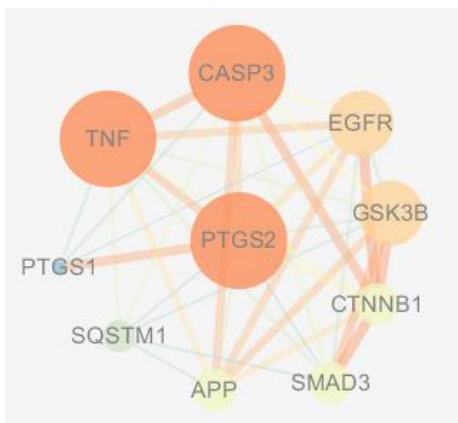


Figure 3: PPI network

3.3. GO enrichment and KEGG enrichment analysis

10 targets were imported into the DAVID database for GO and KEGG enrichment analysis. By screening out $P < 0.05$, the GO function items were divided into 94 biological process (BP), 10 Cellular component (CC) and 21 Molecular function (MF) items. Screening before 10, respectively, using microscopic letter online mapping software (<http://www.bioinformatics.com.cn/>) make analysis diagram, as shown in Figure 4. GO functional analysis is divided into three parts. In BP, it mainly involves apoptosis reaction, nitric oxide biosynthesis, protein phosphorylation, peptide serine phosphorylation and so on. CC mainly involves cytoplasm, nucleus, cell membrane, etc. CC mainly involves peroxidase synthase activity, β -catenin and protein kinase binding in prostaglandins.

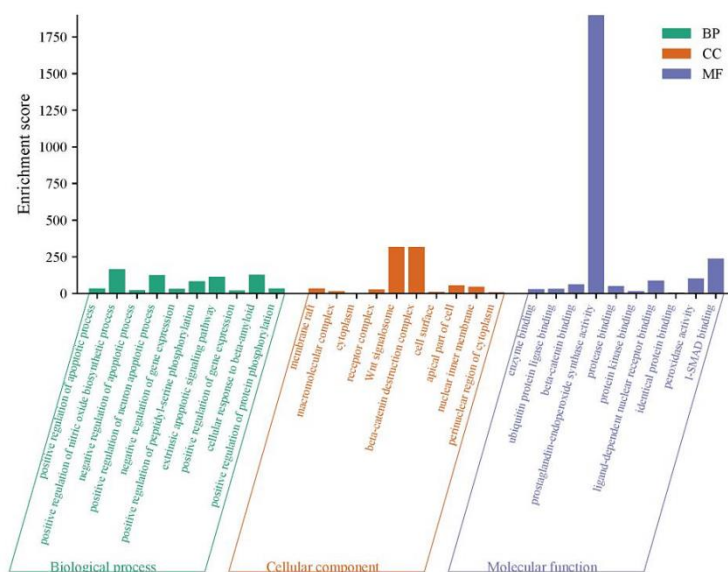


Figure 4: GO functional enrichment analysis

KEGG enrichment analysis was obtained, and 34 signaling pathways were obtained after $P < 0.05$ screening. The first 20 KEGG signaling pathways were selected for visual analysis on microbiosignals as well, as shown in Figure 5. KEGG enriched target genes mainly showed 20 signaling pathways, including neurodegenerative disease pathways (7 targets, 70%), human cytomegalovirus infection (6 targets, 60%), and human papillomavirus infection (6 targets, 60%).

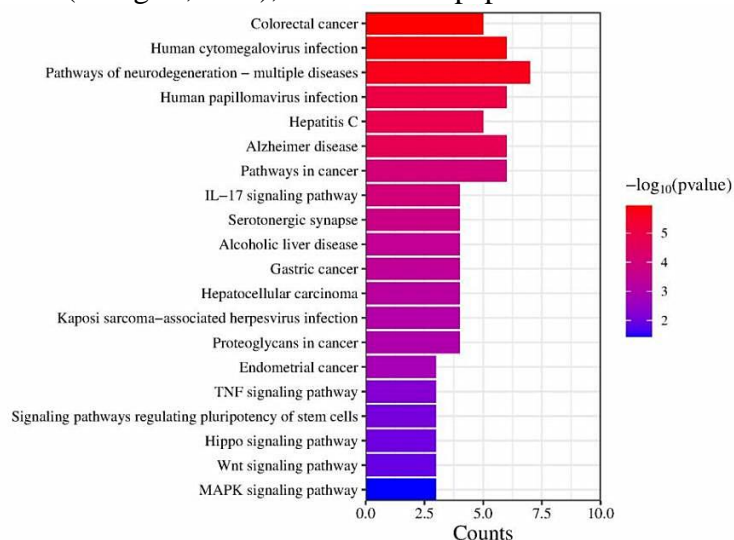


Figure 5: Analysis of KEGG enrichment pathway

3.4. Construct a "drug-target-pathway-disease" network analysis diagram

Based on the 10 intersection targets of CTS and KBD and the screening out of the KEGG enrichment pathway associated with KBD, the "drug-target-pathway-disease" network was constructed via cytoscape, as shown in Figure 6. There are 19 nodes and 41 edges. The higher the Degree, the darker the color, the larger the graph. The "drug-target-pathway-disease" network diagram clearly shows that CTS can treat KBD with multiple targets and multiple pathways.

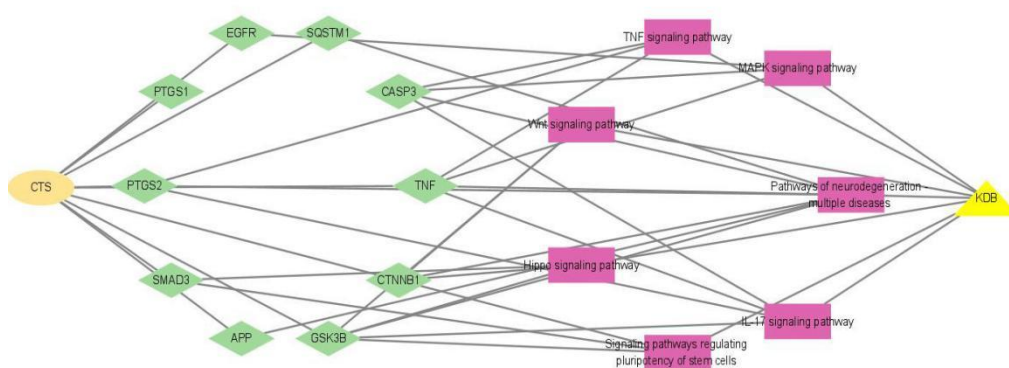


Figure 6: Network analysis diagram of "drug-target-pathway-disease"

4. Discussion

From the analysis of PPI network, the Degree values of PTGS2, TNF and CASP3 ranked the top three. TNF is mainly secreted by macrophages [10], which can induce the generation and spread of many inflammations, and even cause the metabolic disorders of chondrocytes [11], leading to

cartilage destruction, which may exacerbate the development of KBD. Prostaglandin peroxidase synthase 2 is a key enzyme in prostaglandin biosynthesis, and non-steroidal anti-inflammatory drugs (NSAID) usually use PTGS2/COX-2 as a pathway of action [12]. NSAID have become the mainstream drugs in the treatment of KBD, playing a huge role in the treatment of KBD [13,14]. Caspase-3 is usually a landmark product of chondrocyte apoptosis, which can evaluate the degree of injury of chondrocytes [15], and the expression of caspase-3 protease can aggravate the apoptosis of chondrocytes.

According to the analysis of the "drug-target-pathway-disease" network diagram, CTS can also regulate the progression of KBD in chondrocytes through Hippo, Wnt and MAPK signaling pathway. Wnt/ β -catenin pathway is involved in different pathophysiological processes of KBD, and overexpression in chondrocytes can lead to cartilage degeneration, chondrocyte hypertrophy and matrix protease expression [16]. In addition to cartilage, synovial membrane and subchondral bone are also target tissues of Wnt pathway, and the injury of these joint structures can aggravate the progression of KBD disease. The Hippo signaling pathway mainly regulates the self-renewal of stem cells, organ morphology and tissue regeneration by regulating cell proliferation and differentiation [17]. The high expression of YAP1 in its downstream can inhibit chondrocyte differentiation. The downregulation of YAP1 is conducive to the maintenance of normal chondrocytes [18]. MAPK signaling pathway plays an important role in macrophage activation and cartilage degradation [19]. Lps-induced polarization of M1 macrophages and iNOS expression are both involved in MAPK signaling pathway [20,21]. Cartilage injury and synovial injury can promote the development of KBD.

In summary, the mechanism of action and feasibility of CTS in the treatment of KBD were explored through network pharmacology, indicating that CTS can treat KBD with multiple targets and multiple pathways, providing certain basis and reference for the treatment of KBD by CTS. However, the above results and speculation are based on relevant databases, which are still updated constantly, and there are algorithm differences among the processing software, which lacks basic experimental verification and needs further experimental verification.

References

- [1] Wang Kewei, Yu Jun, Liu Hui, et al. Endemic Kashin–Beck disease: A food-sourced osteoarthropathy [J]. *Seminars in Arthritis and Rheumatism*, 2020, 50(2):366-372.
- [2] Yu Fangfang, Zuo Juan, Sun Lei, et al. Animal models of Kashin-Beck disease exposed to environmental risk factors: Methods and comparisons [J]. *Ecotoxicology and Environmental Safety*, 2022, 234:113419-113419.
- [3] Fu Guotao, Chen Xueqin, Qi Meng, et al. Status and potential diagnostic roles of essential trace elements in Kashin-Beck disease patients [J]. *Journal of Trace Elements in Medicine and Biology*, 2022, 69:126880-126880.
- [4] Ye Zhiqiang, et al. Expanding the therapeutic potential of *Salvia miltiorrhiza*: a review of its pharmacological applications in musculoskeletal diseases [J]. *Frontiers in Pharmacology*, 2023, 14:1276038-1276038.
- [5] Feng Jinghui, Jung Jeon Sub, Hwang Seung Hwan, et al. The mixture of *Agrimonia pilosa*;Ledeb. and *Salvia miltiorrhiza*;Bunge. extract produces analgesic and anti-inflammatory effects in a collagen-induced arthritis mouse model [J]. *Animal Cells and Systems*, 2022, 26(4):166-173.
- [6] He Jianbo, Li Xiaojuan, Wang Ziyi, et al. Therapeutic Anabolic and Anticatabolic Benefits of Natural Chinese Medicines for the Treatment of Osteoporosis [J]. *Frontiers in pharmacology*, 2019, 10:1344.
- [7] B. Bai, Y. Li. Danshen prevents articular cartilage degeneration via antioxidation in rabbits with osteoarthritis [J]. *Osteoarthritis and Cartilage*, 2016, 24(3):514-520.
- [8] Zhenhua Feng, Wenhao Zheng, Xiaobin Li, et al. Cryptotanshinone protects against IL-1 β -induced inflammation in human osteoarthritis chondrocytes and ameliorates the progression of osteoarthritis in mice [J]. *International Immunopharmacology*, 2017, 50:161-167.
- [9] Luo Tingting, Lu Yuan, Yan Shikai, et al. Network Pharmacology in Research of Chinese Medicine Formula: Methodology, Application and Prospective [J]. *Chinese journal of integrative medicine*, 2020, 26(1):72-80.
- [10] Nie Hong, Zheng Yingxia, Li Runsheng, et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-alpha in rheumatoid arthritis [J]. *Nature medicine*, 2013, 19(3):322-328.
- [11] Wang Tiantian, He Chengqi. Pro-inflammatory cytokines: the link between obesity and osteoarthritis [J]. *Cytokine*

and Growth Factor Reviews, 2018, 44:38-50.

[12] Arden Nigel K., Perry Thomas A., Bannuru Raveendhara R., et al. Non-surgical management of knee osteoarthritis: comparison of ESCEO and OARSI 2019 guidelines [J]. *Nature Reviews Rheumatology*, 2020, 17(1):59-66.

[13] Roel Nusse, Hans Clevers. Wnt/ β -Catenin Signaling, Disease, and Emerging Therapeutic Modalities [J]. *Cell*, 2017, 169(6):985-999.

[14] Sharma Leena. Osteoarthritis of the Knee [J]. *New England Journal of Medicine*, 2021, 384(1):51-59.

[15] Huang Rongxiang, Hui Zhang, Wei Sun, et al. IRE1 signaling regulates chondrocyte apoptosis and death fate in the osteoarthritis [J]. *Journal of cellular physiology*, 2021, 237(1):118-127.

[16] Wang Cuicui, Shen Jie, Ying Jun, et al. FoxO1 is a crucial mediator of TGF- β /TAK1 signaling and protects against osteoarthritis by maintaining articular cartilage homeostasis [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2020, 117(48):30488-30497.

[17] Consuelo Ibar, Kenneth D. Irvine. Integration of Hippo-YAP Signaling with Metabolism [J]. *Developmental Cell*, 2020, 54(2):256-267.

[18] AbouJaoude A, Courtes M, Badique L, et al. ShcA promotes chondrocyte hypertrophic commitment and osteoarthritis in mice through RunX2 nuclear translocation and YAP1 inactivation [J]. *Osteoarthritis and cartilage*, 2022, 30(10):1365-1375.

[19] Zhang Zhongyin, Yuan Lichan, Liu Yufeng, et al. Integrated cascade nanozyme remodels chondrocyte inflammatory microenvironment in temporomandibular joint osteoarthritis via inhibiting ROS-NF- κ B and MAPK pathways [J]. *Advanced healthcare materials*, 2023, 12(10):e2203195-e2203195.

[20] Lu Jiansen, Zhang Hongbo, Pan Jianying, et al. Fargesin ameliorates osteoarthritis via macrophage reprogramming by downregulating MAPK and NF- κ B pathways [J]. *Arthritis research & therapy*, 2021, 23(1):142-142.

[21] Wang Xiaolin, Guo Zhou, Lin Jiamin, et al. Indirubin protects chondrocytes and alleviates OA by inhibiting the MAPK and NF- κ B pathways [J]. *International immunopharmacology*, 2022, 115:109624-109624.