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Exploration on the Mechanism of Action of Qingbai Powder in the Treatment of Chronic Soft Tissue Injury Based on Network Pharmacology

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Abstract: *This study aimed to systematically explore the potential mechanism of action of Qingbai Powder (QBP) in treating chronic soft tissue injury (CSTI) based on network pharmacology. Active ingredients and their corresponding targets of the herbal components in QBP were screened by integrating the TCMSP database. CSTI-related targets were retrieved from the GeneCards disease database. A "compound-target" interaction network was constructed. Protein-protein interaction (PPI) network analysis, Gene Ontology (GO) functional enrichment analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were employed to elucidate the biological functions of the core targets and associated signaling pathways. Resultly, a total of 35 active constituents of QBP and 136 potential therapeutic targets for CSTI were identified. The core active compounds were quercetin, kaempferol, beta-sitosterol, and stigmasterol. The core targets included AKT1, IL6, TNF, TP53, IL1B, CASP3, EGFR, PTGS2, ESR1, and MMP9. Enrichment analysis revealed that the core pathways were primarily enriched in the TNF signaling pathway, IL-17 signaling pathway, and NF-κB signaling pathway. In conclusion, this study preliminarily reveals the network regulation mechanism by which Qingbai Powder treats CSTI through the synergistic effects of "multi-component, multi-target, multi-pathway," providing a theoretical basis for its further pharmacological research and clinical application.*

Keywords: *Qingbai Powder; Chronic Soft Tissue Injury; Network Pharmacology; Mechanism of Action*

1. Introduction

Soft tissue injury (STI) refers to persistent pathological changes in non-bony tissues such as muscles, tendons, ligaments, and fascia caused by repetitive mechanical stress or overuse [1]. Its clinical manifestations primarily include local pain, swelling, limited joint movement, and tenderness, which significantly impair patients' daily function and quality of life [2]. Based on disease course and etiology, STI can be classified into acute and chronic types. Chronic soft tissue injury (CSTI) generally develops when an acute soft tissue injury is not treated promptly or is improperly managed, and it can also result from chronic strain. Current Western medical treatments often involve non-steroidal anti-inflammatory drugs, physical therapy, or surgical intervention. However, these approaches present limitations such as gastrointestinal side effects, risk of drug dependence, high recurrence rates, and increased economic burden, highlighting the need to explore alternative therapies [3].

In traditional Chinese medicine (TCM) traumatology, soft tissue injury falls under the category of "Shang Jin" (tendon injury), which is primarily attributed to blood stasis obstruction. The key pathogenesis involves blood stasis and qi stagnation, leading to disharmony in the collateral vessels. In recent years, with the growing acceptance of complementary and alternative medicine systems, Chinese herbal medicine has demonstrated significant advantages in treating soft tissue injuries [4,5].

Qingbai Powder (QBP), composed of Atractylodis Macrocephalae Rhizoma (Baizhu), Paeoniae Radix Alba (Baishao), Angelicae Dahuricae Radix (Baizhi), Angelicae Sinensis Radix (Danggui), and Notoginseng Radix (Sanqi), is an empirical formula developed by Professor Zheng Huaixian from Sichuan Provincial Orthopedic Hospital. For decades, this formula has been widely used by numerous medical institutions and professional sports teams, demonstrating remarkable efficacy in treating CSTI and playing a crucial role in managing sports injuries. Preliminary studies on QBP in animal models of

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CSTI have shown that it inhibits the overexpression of Collagen-I and Collagen-III after skeletal muscle injury, thereby preventing scar formation in the injured muscle tissue [6,7]. However, its pharmacodynamic material basis and molecular-level mechanisms of action have not yet been systematically elucidated.

Network pharmacology serves as an effective method for studying classical TCM formulas or new drug development and can provide a foundation for research and development of hospital preparations [8]. Therefore, this study employs a network pharmacology approach to predict the material basis and mechanism of action of QBP in treating CSTI, aiming to provide a reference for the development of hospital preparations.

2. Materials and Methods

2.1 Collection of QBP Active Ingredients and Target Prediction

The active ingredients and corresponding targets of the five herbal medicines comprising QBP (Atractylodis Macrocephalae Rhizoma (Baizhu), Paeoniae Radix Alba (Baishao), Angelicae Dahuricae Radix (Baizhi), Angelicae Sinensis Radix (Danggui), and Notoginseng Radix (Sanqi)) were predicted using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP: http://tcmspw.com/tcmsp.php). Screening criteria were set as oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 to identify the effective active ingredients and their targets. All obtained compound targets were integrated and deduplicated, and the target gene names were standardized using the Uniprot database (https://www.uniprot.org/).

2.2 Acquisition of CSTI-Related Targets

The GeneCards database (https://www.genecards.org/) was comprehensively searched using the keyword "chronic soft tissue injury". Targets with a Relevance score greater than or equal to the median value were selected as disease-related targets.

2.3 Identification of Intersecting Targets for the Drug and Disease

The obtained QBP component targets and CSTI disease targets were mapped against each other. The Jvenn online platform (https://www.bioinformatics.com.cn/static/others/jvenn_en/example.html) was used to identify the intersecting target genes.

2.4 Construction of the Herb-Active Ingredient-Disease-Target Network and Screening of Key Active Ingredients

Files named "network.xlsx" and "type.xlsx", containing the drug components and the aforementioned intersecting targets, were prepared and imported into Cytoscape 3.10.1 to construct a Herb-Active Ingredient-Disease-Target network. Network topology analysis was performed, and the key active ingredients of QBP for treating CSTI were screened based on the Degree value.

2.5 Construction of the Protein-Protein Interaction (PPI) Network and Screening of Key Targets

To further investigate the protein-protein interactions underlying QBP's treatment of CSTI, the intersecting genes between the drug's core components and the disease were uploaded to the STRING database (https://string-db.org/) to construct a PPI network. The species was set to "Homo sapiens", the minimum required interaction score was set to 0.4, and disconnected nodes were hidden in the network. Other parameters retained their default settings. The result was saved in TSV format. The TSV file was imported into Cytoscape 3.10.1 for network topology analysis. Based on the network analysis results, key targets were screened using the Degree value.

2.6 GO and KEGG Pathway Enrichment Analysis

To gain deeper insight into the potential mechanism of action of QBP in treating CSTI, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the intersecting targets of the drug action targets and the disease-related targets. The intersecting targets were uploaded to the DAVID database (https://david.ncifcrf.gov/summary.jsp) for

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visualization. The gene identifier was set to OFFICIAL_GENE_SYMBOL, and the species was specified as Homo sapiens. GO functional annotation analyzed the role of the drug's target proteins in gene function from three aspects: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The top 10 entries for BP, CC, and MF were selected based on enrichment score. The top 20 KEGG pathway entries most relevant to the disease were selected as key signaling pathways for the drug's therapeutic action, predicting the mechanism of action. Finally, visualization analysis was conducted using the bioinformatics online platform (http://www.bioinformatics.com.cn/).

3. Results

3.1 Collection of QBP Active Ingredients and Prediction of Drug and Disease Targets

A total of 35 active compounds were screened from the TCMSP database. After standardizing the target gene names using the Uniprot database, 174 potential drug targets were obtained. Retrieval from the GeneCards database identified 3295 targets related to CSTI. Drug-disease target intersection analysis identified 136 common targets (Figure 1), suggesting these as the potential therapeutic targets of QBP for treating CSTI.

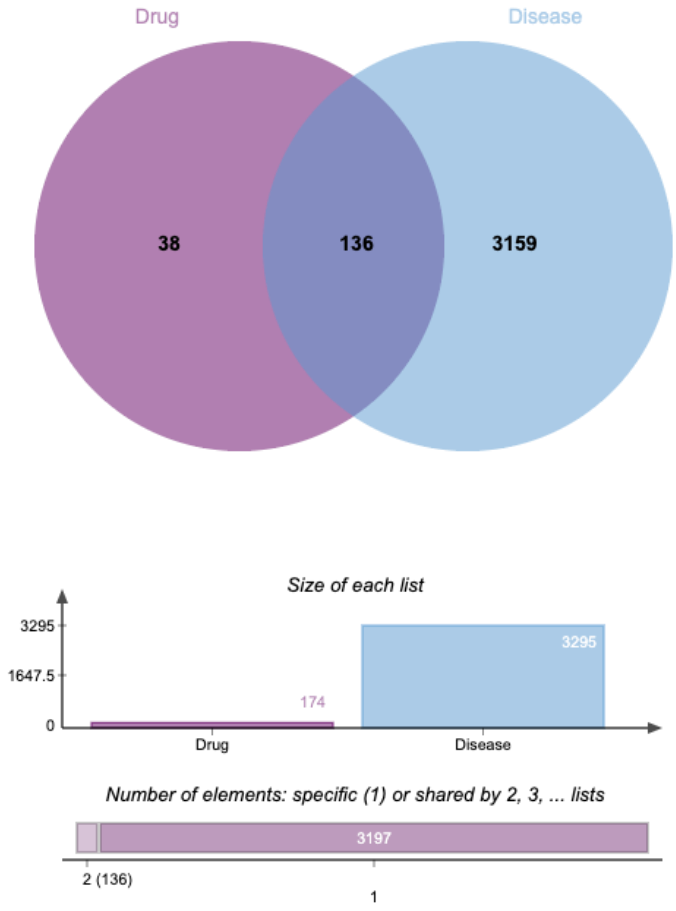


Figure 1: Venn diagram of overlapping targets between the drug (QBP) and disease (CSTI)

3.2 Construction of the "Herb-Active Ingredient-Disease-Target" Network and Identification of Key Active Ingredients

Based on the corresponding relationships between active ingredients and intersecting targets, an interaction network comprising 184 nodes and 573 edges was constructed using Cytoscape 3.10.1 (Figure 2). Network topology analysis, ranked by Degree value, identified the top five compounds as key active ingredients: quercetin, kaempferol, beta-sitosterol, 14-acetyl-12-senecioyl-2E, 8Z, 10E-atractylentriol, and stigmasterol. These components are likely to play crucial roles in the therapeutic mechanism of QBP against CSTI.

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415

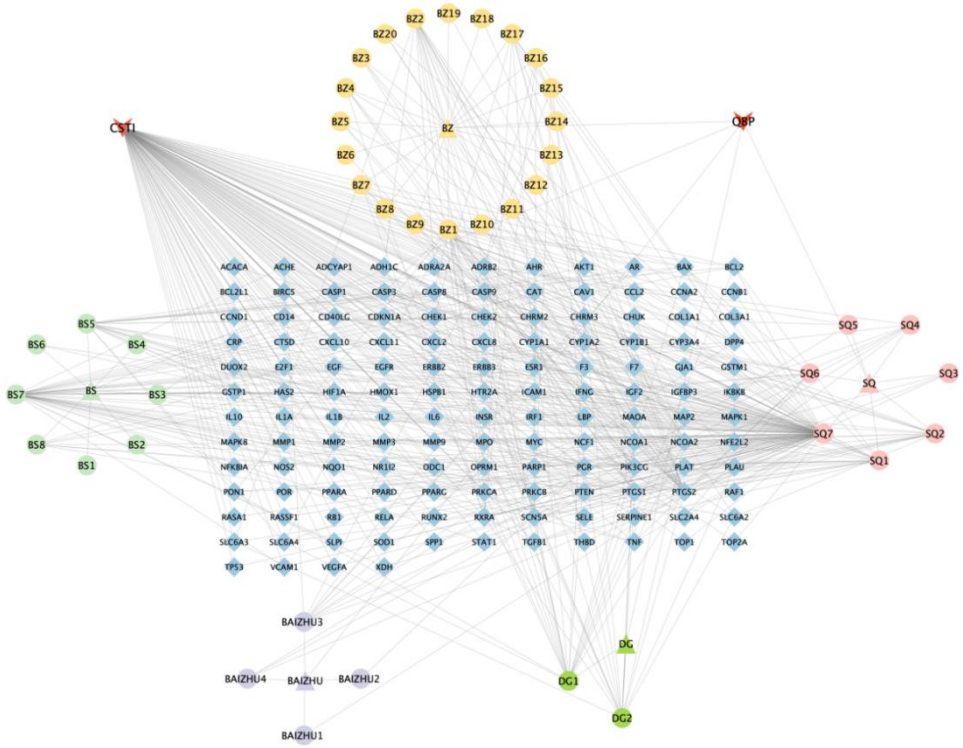


Figure 2: Herb-active ingredient-disease-target interaction network.

Note: In the network diagram, triangles represent herbal medicines, circles denote active ingredients, diamonds indicate targets corresponding to the ingredients, and inverted triangles (V-shapes) represent the formula name and disease name.

3.3 Protein-Protein Interaction (PPI) Network Construction and Core Target Screening

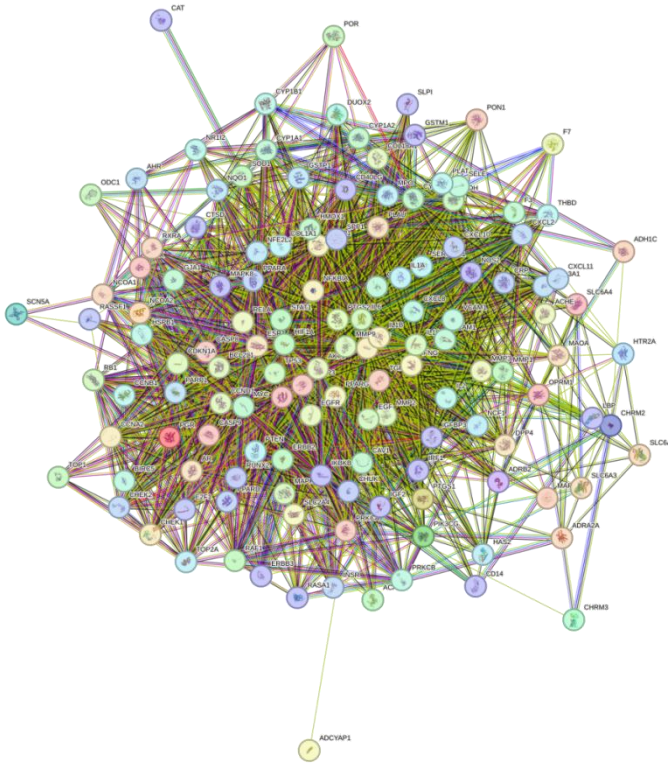


Figure 3: Preliminary PPI network from the STRING database

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415

A preliminary PPI network of the 136 intersecting targets was constructed using the STRING database (Figure 3). Visualization and analysis in Cytoscape yielded a final interaction network comprising 136 nodes and 2, 813 edges (Figure 4). Network topology analysis, applying a Degree value threshold of ≥90, identified the top 10 core targets: AKT1, IL6, TNF, TP53, IL1B, CASP3, EGFR, PTGS2, ESR1, and MMP9 (Figure 5, Table 1). These targets are considered to play a pivotal role in the network regulatory mechanism.

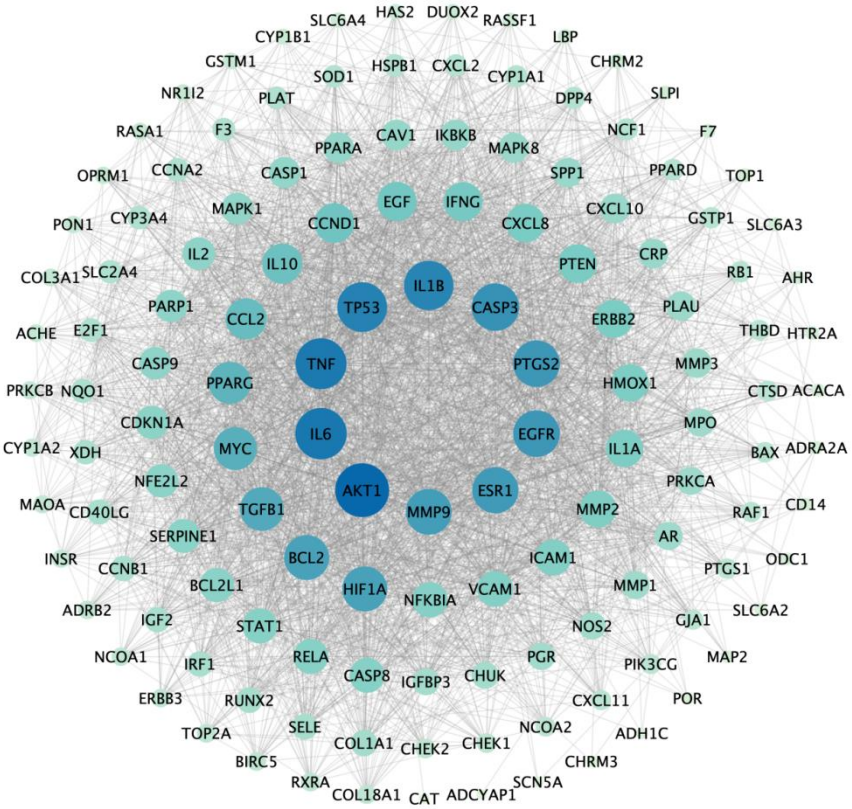


Figure 4: Protein-protein interaction (PPI) network diagram

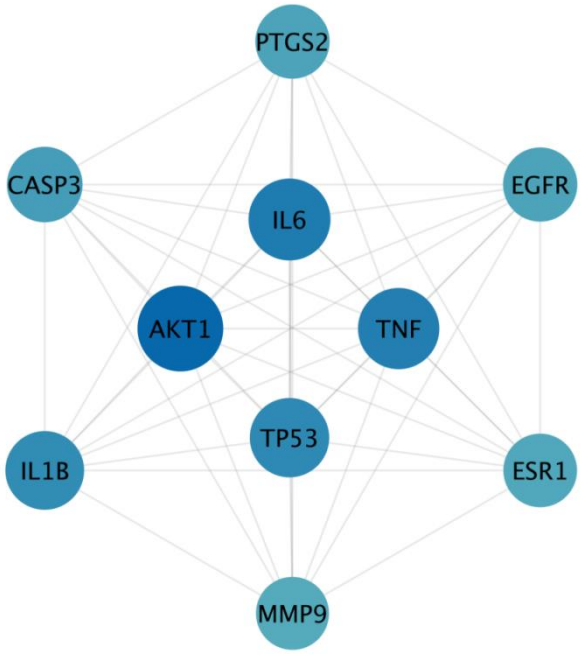


Figure 5: Protein-protein interaction (PPI) network of core targets

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415

Table 1: Topological analysis parameters of the PPI network

Target	Degree	BC	CC
AKT1	110	0.053509412	0.838509317
IL6	104	0.042346449	0.813253012
TNF	103	0.035379487	0.808383234
TP53	100	0.034832904	0.794117647
IL1B	99	0.035457611	0.789473684
CASP3	94	0.022664423	0.762711864
EGFR	92	0.024202911	0.754189944
PTGS2	92	0.031134981	0.758426966
ESR1	91	0.038333626	0.754189944
MMP9	90	0.0169396	0.745856354

3.4 Biofunctional Enrichment Analysis

3.4.1 GO Functional Enrichment Analysis

GO enrichment analysis of the drug-disease intersecting genes identified a total of 2, 621 significant entries ($P < 0.05$). This included 2, 343 entries in Biological Process (BP), primarily involved in response to lipopolysaccharide, response to molecule of bacterial origin, response to xenobiotic stimulus, response to oxygen levels, and response to hypoxia. For Cellular Component (CC), 87 entries were significantly enriched in structures such as membrane raft, membrane microdomain, caveola, plasma membrane raft, and vesicle lumen. Regarding Molecular Function (MF), 191 entries were identified, with key involvements in DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, cytokine receptor binding, cytokine activity, and transcription coactivator binding. Figure 6 displays the top 10 most significant entries ranked by P-value from each of the three categories.

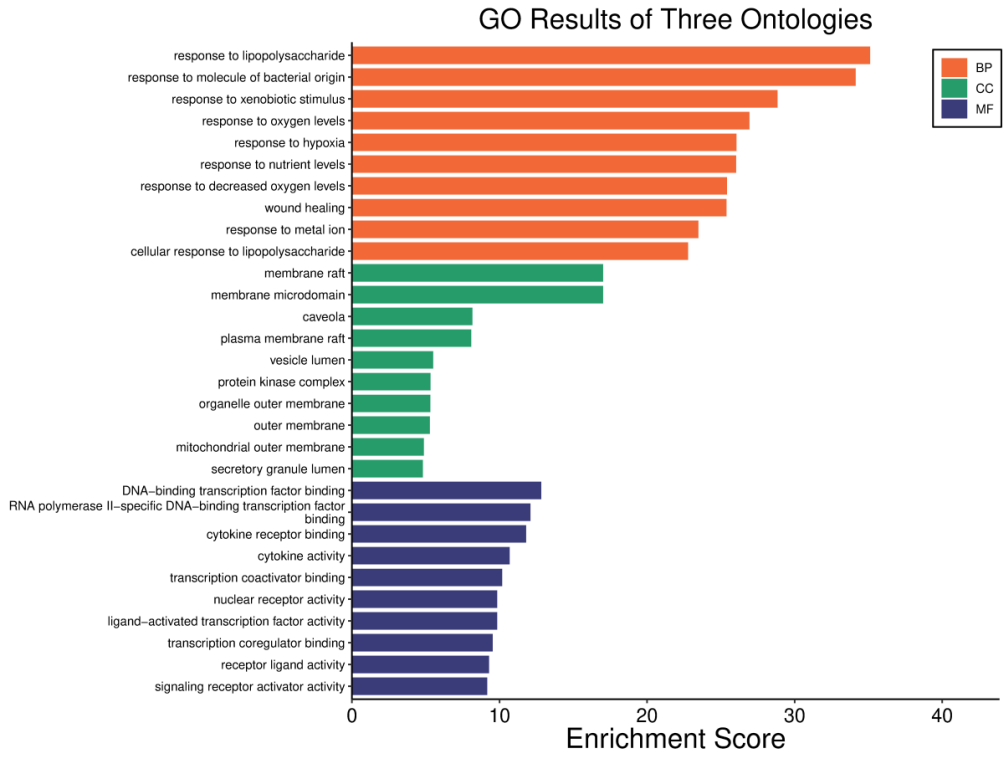


Figure 6: Top 10 entries of GO functional enrichment analysis

3.4.2 KEGG Pathway Enrichment Analysis

KEGG pathway analysis of the intersecting targets identified 179 significantly enriched pathways ($P < 0.05$). Figure 7 displays the top 20 core pathways ranked by P-value, which included the TNF signaling pathway, IL-17 signaling pathway, and NF-kappa B signaling pathway, among others. The mechanism

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415

of action involving these key signaling pathways is illustrated in Figure 8.

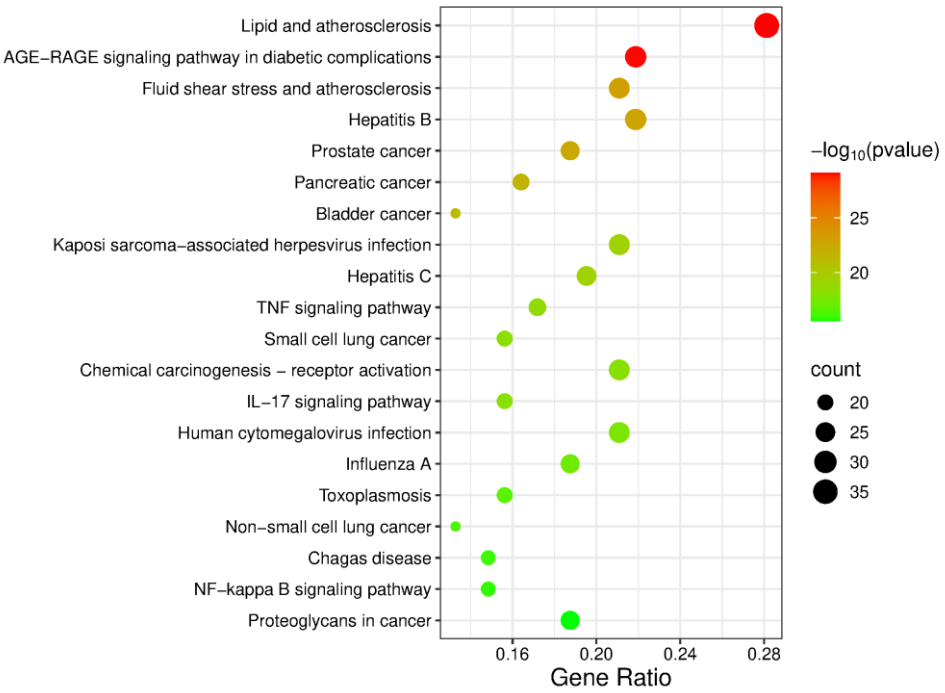
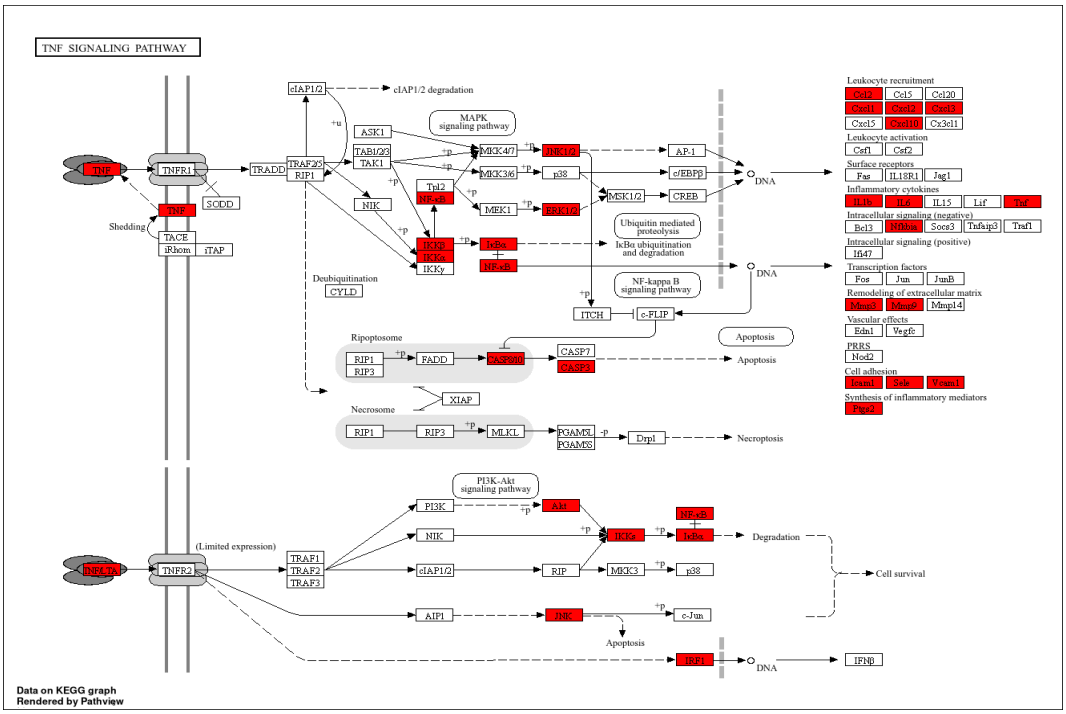
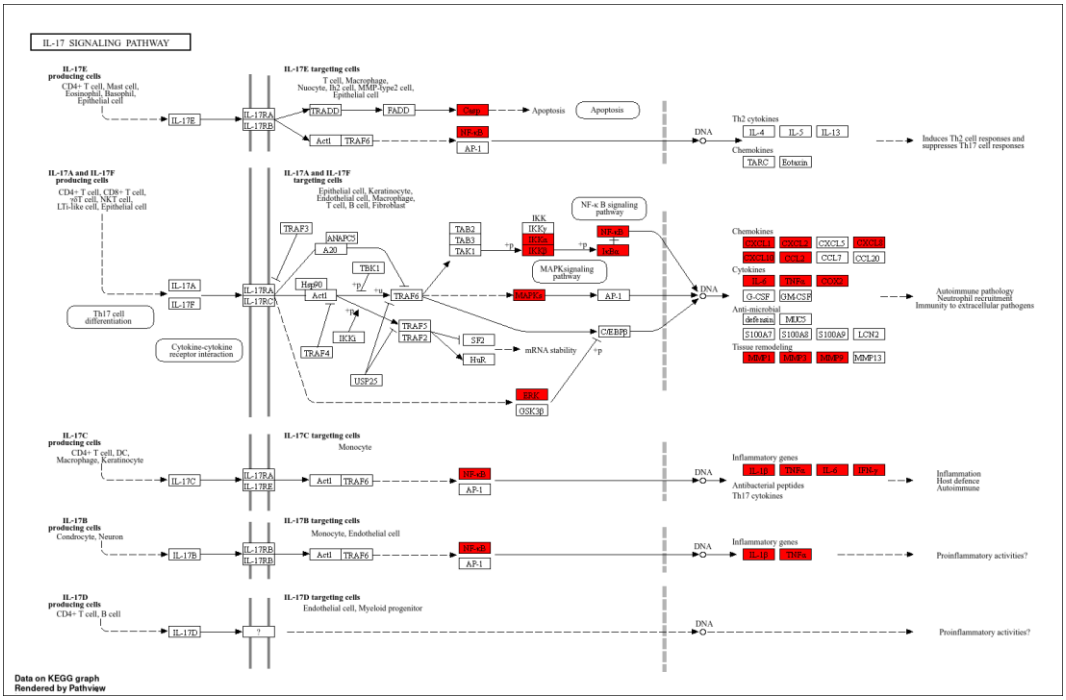


Figure 7: Top 20 entries of KEGG pathway enrichment analysis

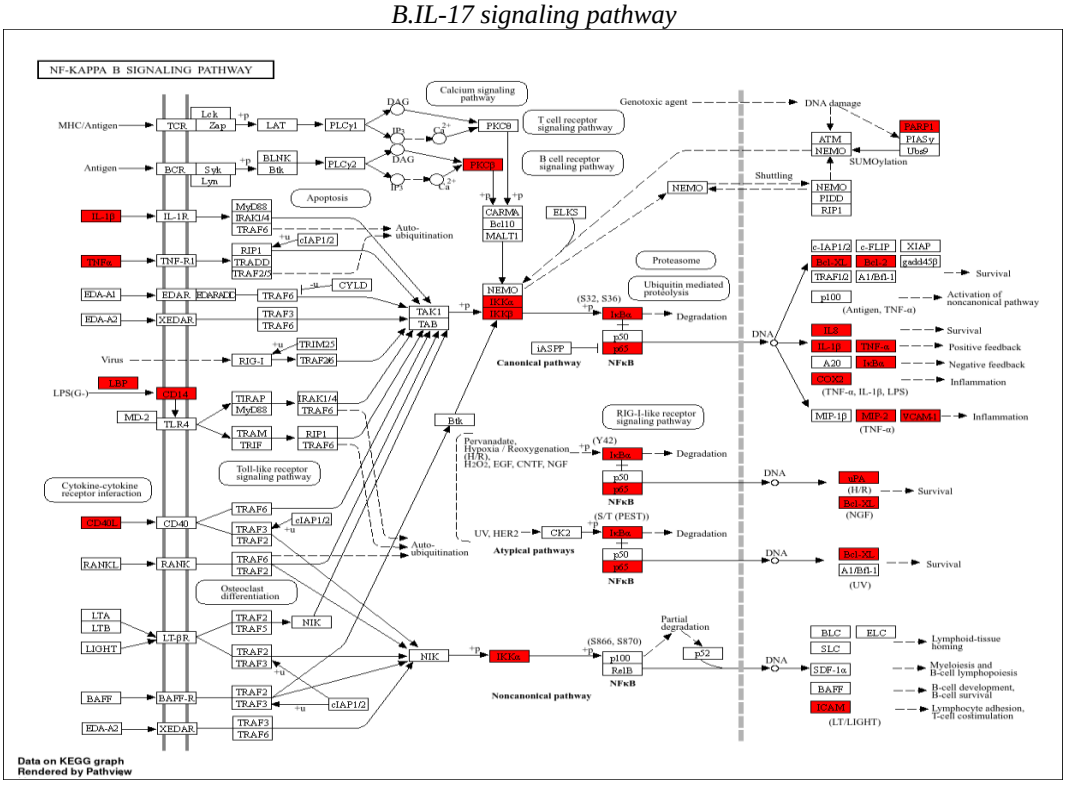


A.TNF signaling pathway

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415



B.IL-17 signaling pathway



C.NF-kappa B signaling pathway

Figure 8: Mechanism of action of the core signaling pathways. Core targets are highlighted in red

4. Discussion

CSTI is a prevalent clinical condition that severely impacts patients' physical and mental well-being as well as functional capacity. In TCM theory, CSTI is classified under “tendon injury,” with a core pathogenesis rooted in mechanical strain or internal deficiency leading to dysfunction in qi and blood circulation, ultimately forming the pattern of “qi stagnation and blood stasis.” This further results in meridian obstruction and malnourishment of tendons and vessels, clinically manifested as localized swelling, pain, functional impairment, and even muscle spasms. QBP is designed based on the principles

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of "activating blood circulation to resolve stasis, promoting qi flow to relieve pain, and nourishing blood to strengthen tendons." In this formulation, Sanqi serves as the monarch component by resolving stasis, stopping bleeding, invigorating blood, and alleviating pain; Danggui acts as the minister component by nourishing and activating blood, unblocking collaterals, and relieving pain, thereby enhancing the stasis-resolving effect and addressing deficiency; Baishao and Baizhu function together as assistant components—Baishao nourishes blood and soothes the liver to ease tension and pain, while Baizhu strengthens the spleen, boosts qi, dries dampness, and promotes diuresis, thereby supporting the generation of qi and blood and enhancing dampness elimination and tendon relaxation; Baizhi serves as the guiding component, with its properties of dispelling wind and cold, reducing swelling, relieving pain, and directing all ingredients to the affected area. Collectively, the formula works to break stasis, unblock collaterals, simultaneously regulate qi and blood, and address both the root and branch of CSTI.

Based on network pharmacology analysis, this study preliminarily identified components such as quercetin, kaempferol, beta-sitosterol, 14-acetyl-12-senecioyl-2E, 8Z, 10E-atractylentriol, and stigmasterol as potential core active substances of QBP in treating CSTI. Among them, beta-sitosterol and stigmasterol are common constituents shared by Sanqi, Danggui, Baishao, and Baizhi, indicating that the therapeutic effect of the formula results from multi-component and multi-target synergism, which can be attributed to the compatibility of multiple herbs. Studies have shown that quercetin exerts anti-inflammatory, anti-edema, and analgesic effects by inhibiting oxidative stress, downregulating COX-2 expression, reducing proteoglycan degradation, and activating the Nrf2/HO-1 signaling pathway, thereby significantly suppressing key pro-inflammatory factors such as TNF-α, IL-1β, IL-17, and MCP-1 [9]. Kaempferol modulates both the NF-κB and MAPK signaling pathways, simultaneously inhibiting osteoclast differentiation and promoting osteoblast activity, thereby exerting dual regulatory functions in anti-inflammation and tissue repair [10]. Beta-sitosterol exerts anti-inflammatory effects by suppressing NLRP3 inflammasome activation in epidermal cells and macrophages, subsequently inhibiting Caspase-1 production and MAPK pathway phosphorylation, which leads to reduced expression of inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 [11]. Stigmasterol significantly inhibits LPS-induced mRNA and protein expression of COX-2 and iNOS, as well as the release of PGE2 and NO, thereby effectively blocking the inflammatory cascade [12]. These components form a multi-dimensional synergistic network within the "inflammation-oxidation-repair" axis, collectively constituting the pharmacological basis of QBP in the treatment of CSTI.

PPI analysis revealed that the key targets of QBP in treating CSTI primarily include AKT1, IL6, TNF, TP53, IL1B, CASP3, EGFR, PTGS2, ESR1, and MMP9. Among these, AKT1 serves as a critical node in the PI3K/AKT signaling pathway, whose phosphorylation can inhibit apoptosis and promote fibroblast proliferation and angiogenesis, playing a vital role in soft tissue repair [13]. IL6, TNF, and IL1B form a core inflammatory cytokine network, wherein TNF activates the NF-κB pathway and induces substantial expression of IL6 and IL1B, collectively driving local inflammatory cascades and pain sensitization. Meanwhile, IL1B can also promote the expression of matrix metalloproteinases, accelerating extracellular matrix degradation [14,15]. TP53 exhibits a dual regulatory role in the soft tissue injury microenvironment: on one hand, it inhibits abnormal proliferation by inducing cell cycle arrest; on the other hand, it promotes apoptotic signals under excessive oxidative stress. Dysregulation of its expression may delay tissue repair [16].

Further GO and KEGG enrichment analyses indicated that QBP exerts its therapeutic effects on CSTI primarily through key pathways such as the TNF signaling pathway, IL-17 signaling pathway, and NF-kappa B signaling pathway. TNF can interact with various other cytokines to participate in inflammatory responses. NF-κB mediates the expression of several inflammatory genes, such as iNOS, COX-2, and chemokines, and also contributes to the production of matrix metalloproteinases including MMP-1, MMP-9, and MMP-13, while feedback-regulating multiple inflammatory mediators, including TNF-α [17]. IL-17 can induce cartilage, synovial cells, macrophages, and bone cells to secrete pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [18].

5. Conclusion

In summary, QBP treats CSTI through multi-component, multi-target, and multi-pathway mechanisms. The therapeutic mechanism likely involves active components such as quercetin, kaempferol, beta-sitosterol, and stigmasterol targeting key entities like AKT1, IL6, TNF, TP53, and IL1B, as well as modulating pathways including the TNF, IL-17, and NF-kappa B signaling pathways, thereby inhibiting inflammatory responses and regulating apoptosis to achieve anti-inflammatory and analgesic effects. Future research should focus on further elucidating the mechanisms of QBP in treating CSTI

ISSN 2706-6819 Vol.7, Issue 4: 118-127, DOI: 10.25236/IJFM.2025.070415

from the perspective of inflammatory factors and signaling pathways.

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