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A Network Pharmacological Study of Qianjin Sanhuang Decoction in the Treatment of Osteoarthritic Knee Disease

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Abstract: ***Objective:*** *To predict the action targets of Qianjin Sanhuang decoction in the treatment of knee osteoarthritis through network pharmacology, to explore its potential multi component, multi target, multi, pathway mechanism of action, and to elaborate the relationship between the pharmacological effects of the drug and the disease from the perspective of molecular mechanism.* ***Methods:*** *The Chinese herbal composition of Qianjin Sanhuang decoction was determined by reading ancient texts and reviewing data, and the chemical components of Qianjin Sanhuang decoction were collected through the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP), and the active ingredients of the drug were obtained after oral utilization (OB) and drug-like properties (DL) screening, and the active ingredient target prediction was performed, while the active ingredient targets were retrieved from GeneCards, OMIM, and DisGeNet database to retrieve knee osteoarthritis-related targets. After taking the intersecting targets, a software called cytoscape 3.9.0 was used for mapping, with the goal of constructing a component-target network map, followed by a string database for mapping, with the goal of making a network map of target protein-target protein interactions, and finally, a gene ontology (GO) enrichment analysis and a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, this analysis process was performed in the metascape database.* ***Results:*** *A total of 1272 targets related to osteoarthritis of the knee were collected in this study, 89 active ingredients of Qianjin Sanhuang decoction, involving 267 targets, and 121 therapeutic targets involving osteoarthritis of the knee, and the results of GO enrichment analysis showed that it involved the response to inorganic substances, positive regulation of cell migration, response to oxygen content, response to lipopolysaccharide, response to peptides, regulation of cell adhesion, response to extracellular stimuli, negative regulation of cell differentiation, positive regulation of cell death, glandular development, regulation of apoptotic signaling pathways, development of reproductive structures, response to trauma, response to growth factors, regulation of inflammatory response, cellular response to organic recycling compounds, regulation of proteolysis, etc. After KEGG analysis, the results showed that signaling pathways such as AGE-RAGE pathway and HIF-1 pathway were involved.* ***Conclusion:*** *Qianjin Sanhuang decoction can improve the inflammatory response of knee osteoarthritis through potential multi-pathways, multi-targets and multi-pathways, and can have potential therapeutic effects on osteoarthritis.*

Keywords: Qianjin Sanhuang decoction, Network pharmacology, Osteoarthritis, Core targets, Pathway analysis.

1. Introduction

Osteoarthritis (OA), as a common chronic degenerative disease, has now become the fourth most common disability-causing disease in the world, with the progress of the aging society, the increase in life expectancy, the economic progress, and the improvement of medical care, and the number of the elderly and the senior population accounts for a higher and higher proportion of the incidence of the disease in the population [1]. Knee Osteoarthritis (KOA) is a branch of osteoarthritis, which is mainly characterized by knee pain, joint deformity and mobility disorders. According to statistics, the prevalence of KOA in people over 60 years old in China is as high as 50%, and the prevalence in people over 75 years old is as high as 80%, and the prevalence in women is higher than that in men [2]. The lesion tissue of KOA often involves cartilage, subchondral bone, synovium and periarticular tissues, which may manifest as degeneration of articular cartilage and subchondral bone hyperplasia. At present, the main treatment for KOA is symptomatic treatment, including specific non-steroidal anti-inflammatory analgesic drugs such as celecoxib capsules, which have achieved certain efficacy, but due to individual differences in patients and other factors, there are still some patients with poor efficacy, and can not play a long-term therapeutic role [3]. The application of traditional Chinese medicine (TCM) can provide new ideas and methods for the treatment of OA. It has been found [4] that TCM can play a role in protecting articular cartilage by decreasing inflammatory cytokines and delaying cartilage degeneration.

Qianjin Sanhuang decoction, also known as Zhongjing Sanhuang Tang, has the efficacy of benefiting the qi and relieving the epidermis, removing dampness and dredging the wind, which is from the "Essentials of the Golden Chamber", and then extracted and quoted by the "Preparing for the Emergency Thousand Gold Formulas", which records that it is "for the treatment of stroke, contracture of the hands and feet, pain in the hundreds of joints, annoyance, heat and heart turmoil, malignant cold, and not wanting to eat or drink for a long period of time. "The ancients believed that the Chinese medicine pathogenesis of KOA is the deficiency of the root cause and the external evil, so the treatment is commonly used to dispel the wind and pass the collaterals, remove the dampness and relieve the pain and other therapeutic principles. Clinical studies have shown [5] that Qianjin Sanhuang decoction has a certain degree of efficacy in the treatment of KOA patients, which can alleviate the symptoms of the patients and improve the level of inflammatory factors. Currently, there are fewer reports on the mechanism of Qianjin Sanhuang decoction. In this paper, we will use network pharmacology technology to explore the potential mechanism of action of Qianjin Sanhuang decoction in treating KOA by screening and systematically predicting the potential targets and signaling pathways of Qianjin Sanhuang decoction in treating osteoarthritis of the knee with the aim of

providing theoretical references to the treatment of KOA with traditional Chinese medicine.

2. Information and Methodology

2.1 Collection and Screening of Active Ingredients in Qianjin Sanhuang Decoction

The chemical constituents of all constituent drugs of Qianjin Sanhuang decoction, including ephedra, astragalus, scutellaria baicalensis, duhuo, and xinxin, were retrieved in TCMSP Traditional Chinese Medicine Data Analysis Platform. The preliminary screening conditions were set as Oral Bioavailability (OB) ≥30% and Drug Likeness (DL) ≥0.18 [6]. Duplicate and ineffective ingredients were removed by careful comparison. The results obtained were summarized and the summarized data were queried through drugbank online database (online drug bank) and the nomenclature was standardized through uniprot database.

2.2 Prediction and Screening of Osteoarthritis and Compound-related Targets

Using GeneCards, DisGeNET, TTD (Therapeutic Target Database) and other online databases, we queried the relevance targets of KOA with the keyword "Knee Osteoarthritis", and analyzed the results from GeneCards by taking the median as the cutoff point, and then summarized the results obtained by de-weighting, removing duplicated and false-positive target genes, and obtaining the information of the disease targets of Knee Osteoarthritis.

2.3 Compound-target Network Construction and Analysis

After obtaining the component target and the target of osteoarthritis, using the R language method, we took the intersection target of the two, which is the disease target of the active ingredient of Qianjin Sanhuang decoction acting on KOA, deleted the mismatched active ingredient, and constructed a visual network of compounds-disease targets by using the Cytoscape 3.9.0 network editing and analysis software.

2.4 Protein-protein Interaction Network Construction

In order to find protein targets that are meaningful for disease treatment, protein interactions need to be studied, and for this purpose, a Protein-Protein Interaction (PPI) network needs to be constructed, and the intersecting target proteins were uploaded to the String database, and the confidence score was set to >0.400, and the organism species was set as "Homo sapiens" was used to obtain PPI information after data processing, and the PPI network was drawn by Cytoscape software.

2.5 Enrichment Analysis

The intersecting targets were imported into the Metascape online analysis website, and the research species was set as human, and gene ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Gnomes) enrichment analysis was performed. The content of GO analysis included biological process (BP), molecular function (MF), cellular component (CC) and other data in the treatment of osteoarthritis of the knee by Chijin Sanghwang Tang, and the prediction by enrichment analysis. The GO analysis included biological process (BP), molecular function (MF), and cellular component (CC), etc., and was used to analyze and predict the possible mechanism of the treatment of osteoarthritis of the knee by Chijin Sanhuang Tang. A "pathway-target relationship network" was also constructed to analyze the relationship between pathways and targets of Qianjin Sanhuang decoction for the treatment of osteoarthritis of the knee.

3. Results

3.1 Ingredient Collection and Screening Results of Qianjin Sanhuang Decoction

By using the TCMSP database, the initial screening conditions were DL ≥0.18, OB ≥30%, among which 22 were ephedra, 17 were astragalus, 35 were scutellaria, 7 were Angelica, and 8 were Asarum sieboldii, a total of 95 active ingredients were collected. The basic information of the top 15 DL values is shown in Table 1.

Table 1: Top 15 components of DL values

Compound code	Compound	OB (%)	DL	Drug source
MOL000379	9, 10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92	Astragalus
MOL001458	coptisine	30.67	0.86	Scutellaria
MOL009849	ZINC05223929	31.57	0.83	Asarum
MOL012141	Caribine	37.06	0.83	Asarum
MOL001558	sesamin	56.55	0.83	Asarum
MOL000211	Mairin	55.38	0.78	Astragalus
MOL000033	(3S, 8S, 9S, 10R, 13R, 14S, 17R)-10, 13-dimethyl-17-[(2R, 5S)-5-propan-2-yloctan-2-yl] -2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17-dodecahydro-1H-cyclo penta [a] phenanthren-3-ol	36.23	0.78	Astragalus
MOL002897	epiberberine	43.09	0.78	Scutellaria
MOL001755	24-Ethylcholest-4-en-3-on e	36.08	0.76	Ephedra
MOL000449	Stigmasterol	43.83	0.76	Ephedra
MOL000449	Stigmasterol	43.83	0.76	Scutellaria
MOL000358	beta-sitosterol	36.91	0.75	Ephedra
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	Ephedra
MOL000296	hederagenin	36.91	0.75	Astragalus
MOL000359	sitosterol	36.91	0.75	Scutellaria

3.2 Target Screening

The targets of each component of Qianjin Sanhuang decoction were screened by Drugbank database, including 497 Ephedra, 461 astragalus, 506 scutellaria, 92 Angelica, 164 Asarum sieboldii, a total of 267 targets were collected by removing duplicate targets. The relevant indexes were searched with "Knee Osteoarthritis" as keywords through GeneCards, TTD, DisGeNet and DrugBank databases. The median of 1.38 was taken as the cut-off point according to score in GeneCards, a total of 1159 targets were obtained, 80 targets were collected by DrugBank, 200 KOA-related targets were obtained from DisGeNET database and 26 KOA-related targets were obtained from TTD, a total of 1272 osteoarthritis-related targets were obtained by removing repetitive targets, and 121 osteoarthritis-qianjin Sanhuang decoction targets were

obtained by R language mapping. See Figure 1

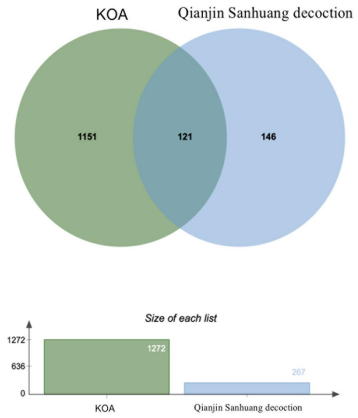

Figure 1: Intersection target of Qianjin Sanhuang decoction-knee osteoarthritis

3.3 Compounds—Construction and Analysis of Action Target Network

A compound-intersection target network was constructed using Cytoscape software. The network group consisted of 349 nodes with 1522 edges. The degree represented the number of edges connected by the nodes. The results showed that the compounds with the fifth Degree were quercetin (MOL000098 Degree = 286), Kaempferol (MOL000422 Degree = 183), beta-sitosterol (MOL000358 Degree = 77), Stigmasterol (MOL000449 Degree = 60), luteolin luteolin (MOL00006 Degree = 52), the above compounds may play a major role in drug efficacy and therapy. These results suggest that Qianjin Sanhuang decoction can regulate KOA by means of multi-component and multi-target. See Figure 2. The top 15 compounds with Degree values are shown in Table 2.

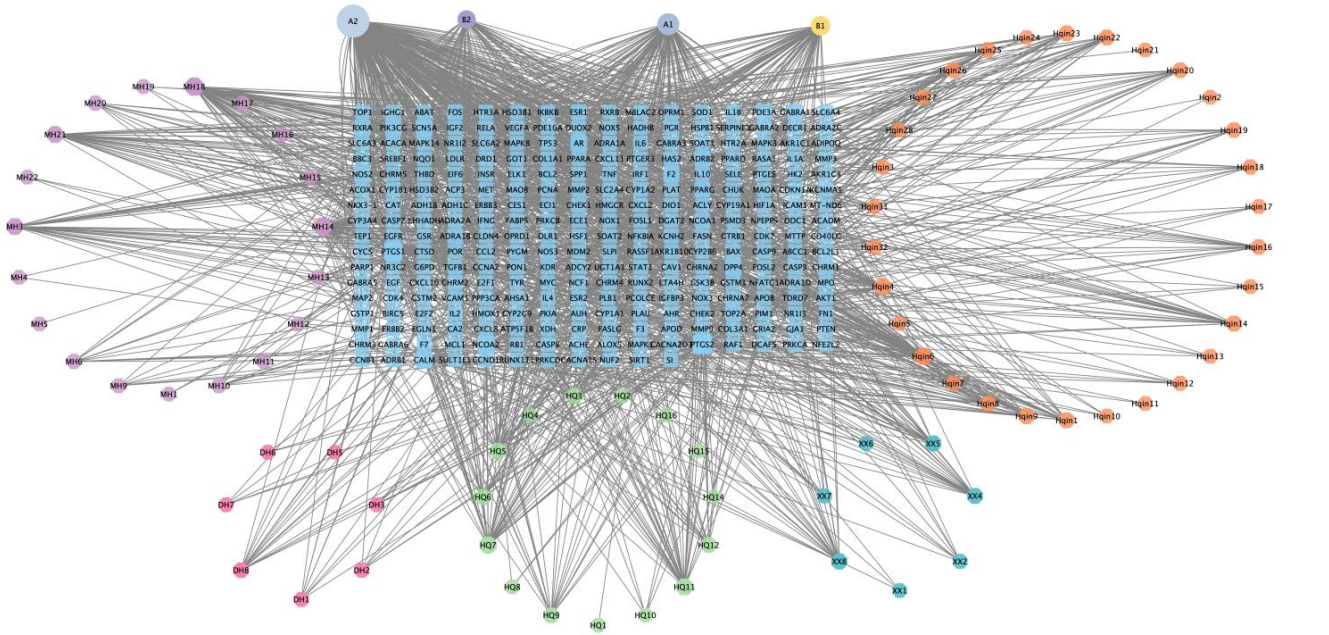

Figure 2: Compound-intersection target

Note: The Square in the center represents the target of the disease, and the five circles formed by the hexagons represent the five traditional Chinese medicines. In order to show the beauty of the data, the chemical composition of the drug was abbreviated as MH (Ephedra), DH (Angelica), HQ (Astragalus), XX (Asarum), Hqin (Scutellaria), followed by the number representing the chemical composition number of the drug, A1, A2, B1, B2 on the top indicate the common components of the traditional Chinese medicine.

Table 2: Node characteristic parameters of the main active components network of Qianjin Sanhuang decoction

Compound	Source of the compound	Compound coding(Mol Name)	Closeness Centrality	Degree	BetweennessCentrality
quercetin	A2(Ephedra, Astragalus)	MOL000098	0.502898551	286	0.428454463
kaempferol	A1(Ephedra, Astragalus, Asarum)	MOL000422	0.411137441	183	0.099330108
beta-sitosterol	B1(Ephedra, Scutellaria, Angelica)	MOL000358	0.38988764	77	0.035790036
Stigmasterol	B2(Ephedra, Angelica)	MOL000449	0.383002208	60	0.047167701
luteolin	MH14(Ephedra)	MOL000006	0.396118721	52	0.086772626
wogonin	Hqin6(Scutellaria)	MOL000173	0.387276786	41	0.049166439
7-O-methylisomucronulatol	HQ7(Astragalus)	MOL000378	0.389013453	40	0.038508765
beta-sitosterol	MH3(Ephedra)	MOL000358	0.387276786	35	0.029813204
formononetin	HQ11(Astragalus)	MOL000392	0.380482456	35	0.044241417
naringenin	MH21(Ephedra)	MOL004328	0.377173913	34	0.09698438
baicalein	Hqin4(Scutellaria)	MOL002714	0.378820961	32	0.061419581
isorhamnetin	HQ5(Astragalus)	MOL000354	0.377995643	31	0.024200369
Cryptopin	XX8(Asarum)	MOL001460	0.373922414	25	0.021713408
acacetin	Hqin9(Scutellaria)	MOL001689	0.371520343	22	0.0164699
3, 9-di-O-methylnissolin	HQ6(Astragalus)	MOL000371	0.373922414	22	0.011028295

3.4 Protein—Protein-protein Interaction Network

The intersection targets were imported into the String database to construct the target Protein Action Network (PPI information) of Qianjin Sanhuang decoction acting on KOA, and the visual PPI network was established by using cytoscape software, the size and color of the target in the graph can be used to reflect the size of the Degree value (number of protein interactions). The larger the node, the larger the Degree value, the color will change from yellow to red, the thickness of the edges and the color depth, used to reflect the size of Combine Score, the larger the value, the thicker the edge, and the darker the color. The results show that there are 125 nodes, 2680 edges, and the average Degree value is 43. There are 11 targets with Degree value greater than 80, which are TNF, AKT1, IL6, il1β, TP53, VEGFA, CASP3, PTGS2, MMP9, HIF1A, CCL2 respectively, see Figure 3, and the data for the first 30 Degree values are shown in Figure 4.

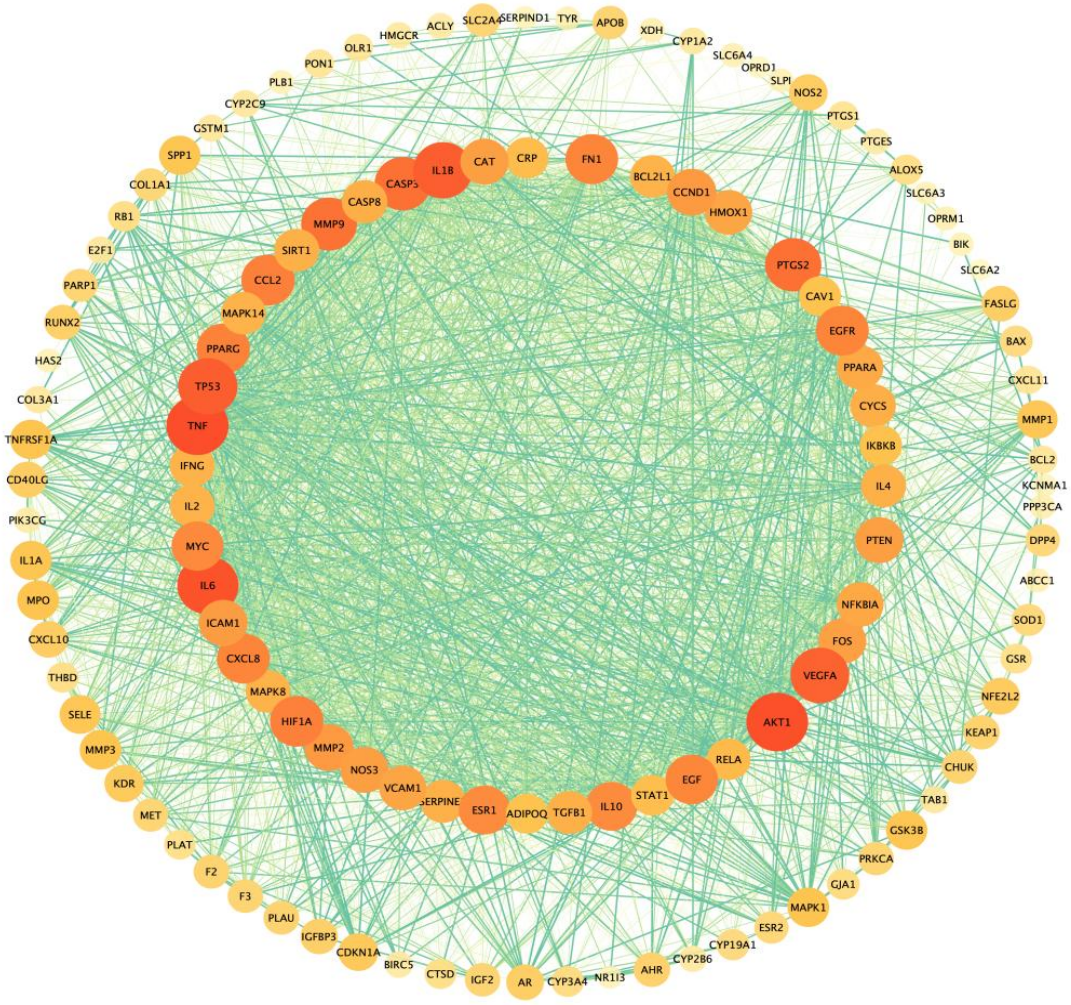

Figure 3: PPI network

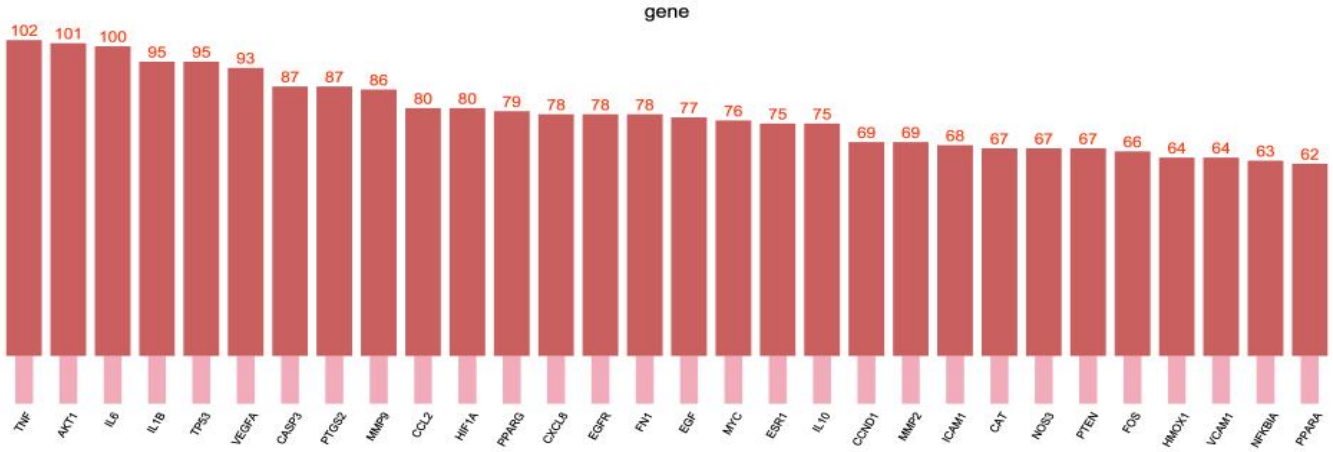

Figure 4: The first 30 Degree targets

3.5 Results of Enrichment Analysis

In order to further clarify the mechanism of Qianjin Sanhuang decoction, and to analyze the biological and signal transduction process of Qianjin Sanhuang decoction on KOA, using the Metascape web site's online analysis function to analyze the intersection targets, the analysis results show that the biological process analysis involves 1730, these include response to inorganic substance, positive regulation of cell migration, response to oxygen levels, response to lipopolysaccharide, regulation of cell adhesion, response to extracellular stimulus, negative regulation of cell differentiation, positive regulation of cell death, regulation of apoptotic signaling pathway, response to growth factor, regulation of inflammatory response, cellular response to organic cyclic compound, regulation of proteolysis, etc., biological function involves 140 items, these include Cytokine receptor binding, protease binding, DNA-binding transcription factor binding, heme binding and monoamine transmembrane transporter activity, the analysis of biological components involved 82 items, these include membrane raft, vesicle lumen, extracellular matrix, extracellular matrix, FICOLIN-1-RICH granule lumen, nuclear envelope and PML body. The KEGG results involved 188 entries, it is involved in AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, pathway in cancer, Chemical carcinogenesis-receptor activation and Relaxin signaling pathway, these results suggest that the active components of Qianjin Sanhuang decoction are distributed in different pathways, and play a regulatory and therapeutic role on KOA through multi-pathway regulation. See Figure 5.According to the results of KEGG analysis, the first 20 main pathway data were imported into cytoscape 3.9.0 software to construct the "Target-pathway" network graph. Qianjin Sanhuang decoction was used to analyze the signal pathway of KOA-related targets. The size of pathway and target was set according to the degree value. The bigger the degree value was, the bigger the shape of the target and pathway was. According to the enrichment analysis of target and pathway, the function of multiple targets is closely related to the generation of KOA. The same target is related to multiple pathways. See Figure 6

Figure 5 Enrichment analysis of potential targets of Qianjin Sanhuang decoction in the intervention of knee osteoarthritis/osteodystrophy (Figure A: Go-bioprocess analysis bar chart; figure B: Go-biocomponent Analysis Bar chart; Panel C: KEGG bubble graph; panel D: GO-biological function analysis bar graph).

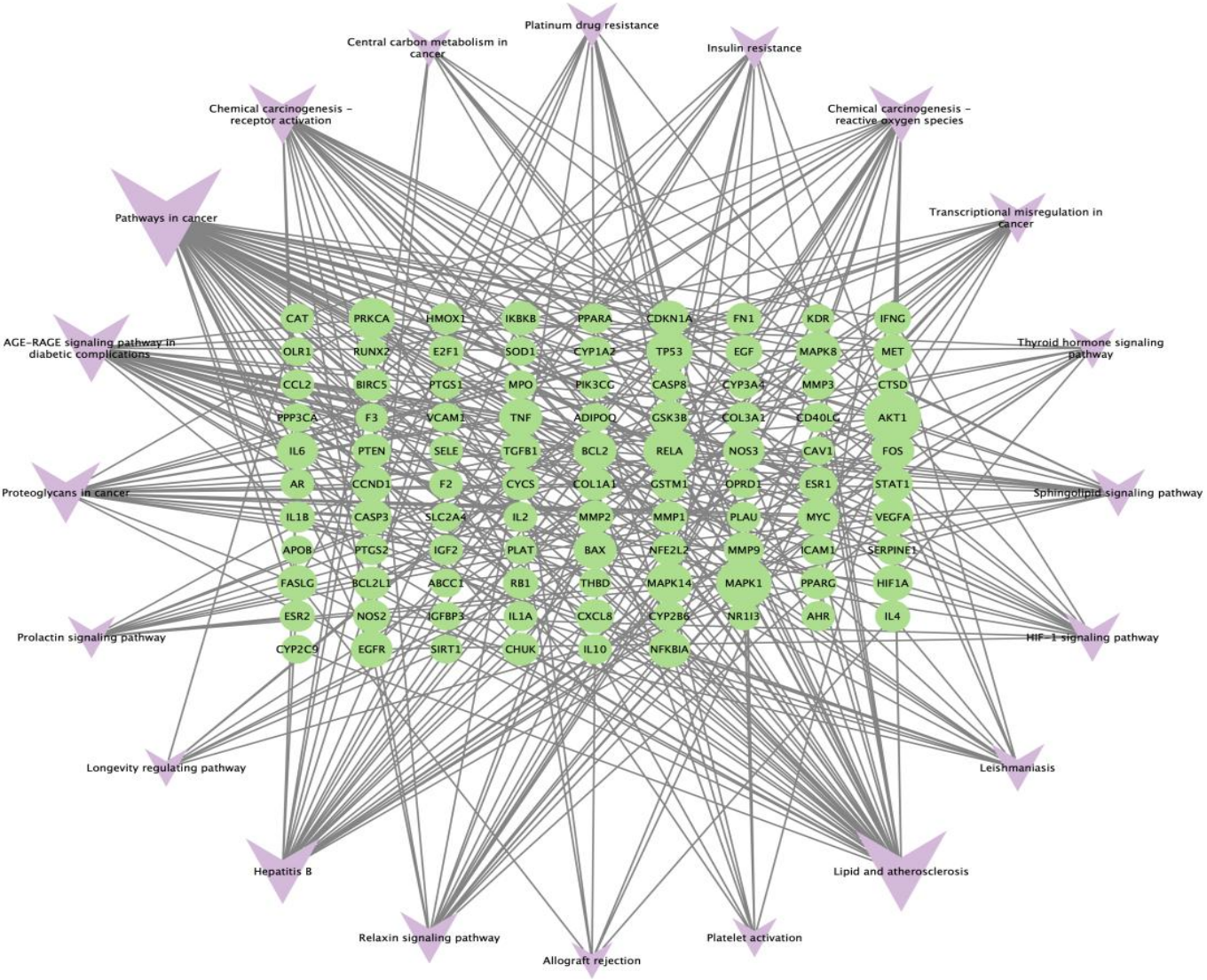

Figure 6: Target-pathway network diagram

3.6 Molecular Docking Screening

The top 6 core components in the Qianjin Sanhuang decoction-KOA target network map obtained from the analysis were molecularly docked with the core targets whose DEGREE values were in the top 6 core targets in the PPI network map, and 6 core targets were selected: TNF, AKT1, IL6, IL1β, TP53, and VEGFA. 6 compounds were selected: quercetin, kaempferol, beta-sitosterol, Stigmasterol, luteolin, wogonin, see Figure 7. It is generally believed that drug molecules with binding energy (Affinity) ≤ -5.0 kJ/mol have better binding activity to the target [7], and the results of the docking showed that the binding activity of the core target with the core components better, the binding images of some components and molecules are shown in Figure 8, from the molecular docking results, it can be seen that the main components of Qianjin Sanhuang decoction can have a certain regulatory effect on KOA.

4. Discussion

Knee osteoarthritis belongs to the category of traditional Chinese medicine "arthralgia", "Question · Arthralgia theory" that is: wind, cold, wet three qi mixed to combine and bi also. The disease is caused by blocking the meridians of the body or internal organs. "Jingui Yaolue" also put forward "calendar festival rheumatism, wet Fu Sheng also... The internal and external call, flow into the joint, pain and can not be flexion-extension." In the scriptures stated: "evil jump into... Low flow waist knee, for failure". "Bi" refers to the obstruction of obstruction. Zhengqi weak, management is not solid, Zhengqi can not overcome evil, invasion of muscle surface meridians and bone nodes, causing body weakness, involving joints, joint or muscle pain, swelling and heavy diseases. Etiology of outside because of exogenous pathogens, the wind cold dampness on tables, because the body is weak, of liver and kidney deficiency in produce of, so the treatment lies in kidney, wind dampness, stop the pain [8]. Modern traditional Chinese medicine (TCM) also called "knee bi", patients with old age, body deficiency, loss of nourishment of muscles and bones, coupled with external exposure to wind, cold and damp pathogens, evil qi into the collaterals, obstruction of qi, muscle and bone disadvantage, so it is manifested as progressive aggravation of chronic pain in the knee joint, Zhangyu etc [9] pointed out that, as a result of KOA patients often long course of disease, the disease in delay no more, so the pathogenesis is often accompanied by qi and blood stasis, poor local knee meridians, blood resistance, qi deficiency and blood supply is insufficient, so the treatment of KOA also shall follow the principle of line fill gas blood treatment. Chen Liming et al [10] pointed out that the onset of KOA is also affected by solar terms. In a research report on 28763 KOA patients, it was found that different syndrome types had different main onset times, which suggested that the treatment should be focused on different directions in different solar terms.

Qianjin Sanhuang Decoction is composed of ephedra, astragalus, scutellaria, angelica and asarum. Scutellaria is bitter and cold, and it is used to treat hot diseases and fire, and heat and poison are abundant. It can clear heat and dry dampness, reduce fire and detoxification, and make the dampness in the body disappear. Astragalus membranaceus GanWen sexual flavour, due to the spleen and lung, can cure table is not solid, the virtual is insufficient, to fill gas wants drug. Ephedra and asarum mainly due to the lung meridian, dissipate cold to solve the surface, dissolve muscles to open the orifices, asarum taste bitter and warm, dispel wind to dissipate cold and relieve pain, and can treat rheumatism Bi pain. The Book of This Classic recorded:" Asarum is used for cough, headache, brain movement, hundred joint spasm, rheumatism and pain, dead muscle. Clear eyes, benefit nine orifices." Ephedra is a kind of hot and warm, which can dissipate cold and benefit water to detumescent and treat wind and cold surface. It is mainly to take its heat and heat diverging nature, the work of dissolving muscle and dispersing, so that the evil of dampness and heat go out, "Bencao Jing Shu" has recorded: "Ephedra..... This medicine is light and clear, so that its accumulation is eliminated, and the pathogen is scattered from the surface. "Angelica can wind to wet, cold check bi. It is mainly used for rheumatism of wind, cold and damp, waist and knee pain. "The pharmaceutical chemical righteousness" records: "angelica, The neck is difficult to relieve, hip and leg pain, otherwise the energy efficiency is also... and the blood medicine, blood circulation and Shu Jin, as miraculous." Clinical studies have found that astragalus membranaceus can regulate the body's immune function, enhance the body's immunity, restore hematopoietic function, and improve the symptoms of qi deficiency and blood stasis in KOA patients. DU et al. [11] found that APS, an active component of astragalus membranaceus, can induce CD4+T cells to produce IL-4 and other factors, thereby increasing the expression of CD8+T cells and exerting immune effects. Zhang et al. [12] found that Astragaloside IV can improve the body's immune response by regulating the activity of CTL and inhibiting the activity of conditional Terg. Scutellaria which contain flavonoids has good anti-inflammatory effects, and the toxicity of peripheral cells, bone marrow cells of human body tiny [13], da-yong xu etc. [14] found that radix scutellariae chemical composition of baicalin can pass regulation exists in the M2 macrophage polarization, STA6 pathways are activated to inhibit the inflammation of rat, relieve spinal cord injury. Wang Min et al. [15] studied the effect of baicalin combined with LPS at different concentrations on the proliferation of mouse lymphocytes and found that it could enhance immunity, which suggested that baicalin may reduce MDA level, remove free radicals, and have good antioxidant capacity. Angelica affects chondrocyte differentiation by participating in calcium signaling pathway, and its component pruchurin can act as an inhibitor to inhibit cyclooxygenase to play an anti-inflammatory role, and its representative agent Duhuo Jisheng decoction has been widely used in clinical practice for the treatment of osteoarthritis [16]. Ban et al. [17] found that imperatorin can inhibit the production of prostaglandins in rat macrophages to play an anti-inflammatory effect, and has different degrees of inhibition on gram-positive bacteria and other fungi. Asarum has better anti-inflammatory analgesic action, its extract asarum methanol can inhibit the pain caused by acetic acid in mice body torsion, found in contrast treatment, its analgesic effect is stronger than aspirin [18], other ingredients such as methyl eugenol by excited GABAA receptor and inhibit the NO receptor levels in order to achieve anti-inflammatory effect [19].

In this study, a total of 267 components of Qianjin Sanhuang

Decoction and 161 potential targets of KOA were screened. The "target-active ingredient" network analysis showed that quercetin, kaempferol, β-sitosterol, sitosterol, luteolin and other compounds may have a high impact on the treatment of diseases. Quercetin flavone, good anti-oxidation, anti-inflammatory effects, delay the disease progression of KOA, quercetin by reducing the proinflammatory factor generated way affect inflammatory signaling pathways, protect the articular cartilage, ensure the microenvironment in the articular cavity balance. Qiu et al. [20] found that the mechanism of action of quercetin on OA rats was reflected in the down-regulation of MMP13 expression and the protection of cartilage. Haleagrahara et al. [21] found that quercetin could reduce the expression of TNF-α, IL-1β and IL-6 in OA mice. Resurrectionlily of phenol as a natural chemical composition has good clinical application effects, the study found that [22], rhizoma kaempferiae phenol can be adjusted by miR - 146 - a way to suppress the cartilage ATDC5 cells inflammatory reaction, and through STAT3 related pathways in order to realize the degradation of the extracellular matrix inhibition [23]. B-sitosterol has certain effects on antibacterial [24], anti-tumor, anti-oxidation [25], anti-inflammation and analgesia, and regulation of bone metabolism. Its anti-inflammatory effect can be reflected in inhibiting the activation of NLRP3 in macrophages, thereby inhibiting the MAPK signaling pathway and reducing the production of inflammatory factors [26]. Zeng Liping et al. [27] found that β-sitosterol can inhibit osteoclast and promote osteogenesis to regulate bone metabolism, and its mechanism is related to promoting estradiol secretion and accelerating estrogen receptor binding in the periosteum. Stigmasterol is a kind of phytosterols. Its antioxidant and anti-inflammatory effects have been confirmed by experiments, and it has good analgesic effect. Bandero et al. [28] found that the mice treated with stigmasterol within 3mg/kg did not produce adverse reactions, and the administration could significantly improve the acute pain after the surgical incision or writhe test. After the pain of sciatic nerve ligation experiment also has obtained the good effect.ANTW1 et al. [29] found that stigmasterol administration can significantly improve the infiltration of inflammatory cells in the epidermis of animal models induced by C48. It has also been found that [30] MMPs can be significantly inhibited after stigmasterol administration in rabbits with OA model. Molecular docking results showed that the core components of Qianjin Sanhuang Decoction, such as quercetin, had good binding activity with the main targets such as NF, AKT1, IL6, IL1β, TP53 and VEGFA, which confirmed the validity of the analysis results to a certain extent and provided a certain reference for future experimental design. The above analysis showed that, The active ingredients of Qianjin Sanhuang Decoction may play a role in the clinical effect through anti-inflammatory, anti-oxidation, analgesia, immune regulation and other directions.

There were 75 overlapping targets between Qianjin Sanhuang decoction and KOA. PPI network analysis showed that the intervention of drugs on TNF, AKT1, IL6, IL1β, CASP3, PTGS2, MMP9, CCL2 and other targets played a key role in treatment, and IL-6 could promote inflammatory differentiation of chondrocytes. Protease secreted metal matrix and promote angiogenesis factor VEGFA, by adjusting the STAT3 and MAPK signal pathways, raised the expression of MMPs, play the role of promoting the growth of blood vessels activation [31]. Il-1β is an important mediator in the regulation of inflammation, which affects the tissue metabolism and degradation of chondrocytes by binding to the IL-1 receptor on the chondrocyte membrane, and its content is positively correlated with the severity of KOA patients [32]. TNF alpha can inhibit rat chondrocytes activity, cause cells to punch rupture, structural damage, apoptosis [33]. Yang Rong et al. [34] pointed out that the content of TNFα in the blood of KOA patients often exceeds the normal level and increases with the severity of the disease. CCl2 is a member of the CC chemokine family, and its mechanism is related to the regulation of the occurrence and development of pain symptoms [35]. MMP9 can accelerate the migration of vascular endothelium and contribute to the repair and generation of blood vessels. However, in KOA, the increased expression level of MMP9 often indicates the progression of KOA lesions. Chondrocytes secrete a large number of MMPs, resulting in disordered cellular environment and severe apoptosis and cartilage destruction [36]. Qianjin Sanhuang Decoction treatment of KOA is mainly manifested in the AGE - RAGE pathway, HIF - 1, relaxin signaling pathways, such as channel, HIF - 1 is the core of the steady state adjust intracellular oxygen transcription factor, the alpha channel HIF - 1 and promote osteoblast bone formation function, improve the fracture healing, increase bone density and bone strength [37], Wang et al. [38] analyzed the effect of HIF-1 on osteogenic differentiation of MSCs by establishing a two-stage cell lineage mathematical model, and the results showed that it stimulated the differentiation rate in a dose-dependent manner.

In conclusion, the same compound of Qianjin Sanhuang Decoction can regulate different targets, and the same target can interfere with different pathways, which reflects its multi-target and multi-pathway intervention form for the disease. Qianjin Sanhuang decoction may play a multi-direction clinical effect through anti-inflammation, anti-oxidation, analgesia, immune regulation and other directions, providing a theoretical basis for its molecular mechanism of treating KOA. It can provide some reference for the follow-up clinical efficacy evaluation index and provide some direction guidance for the follow-up research. The deficiency of this study is that due to the limitations of the network pharmacology method itself, such as the changes of traditional Chinese medicine components in the decoction process and the changes of the dose-effect relationship caused by the different dosage of drugs in the treatment can be used as interference factors. The rationality of setting the screening criteria of TCM needs to be confirmed, the dosage of active components of TCM also affects the clinical efficacy, and the database may not cover the newly discovered targets, so the accuracy needs to be improved. The target prediction algorithm is dependent on different server capacity, specific clinical outcomes needs further experimental verification.

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