Article published online: 2022-07-07

Potential Targets and Molecular Mechanism of Quercetin Against Knee Osteoarthritis

Lingling Li[1] Hailiang Huang[1]

[1] College of Rehabilitation Medicine, Shandong University of Traditional Chinese Medicine, Shandong, China

CMNP 2022;2:e32–e43.

Address for correspondence Hailiang Huang, PhD, College of Rehabilitation Medicine, Shandong University of Traditional Chinese Medicine, 4655 University Road, Ji'nan Shandong 250355, China (e-mail: 60120006@sdutcm.edu.cn).

Abstract

Objective The objective of this study was to clarify the potential mechanism of quercetin against knee osteoarthritis (KOA) based on network pharmacology and molecular docking.

Methods The targets of quercetin were predicted by PubChem and Swiss Target Prediction databases, and the targets of KOA were obtained by DisGeNET, OMIM, and GeneCards databases. Then, the targets of quercetin and KOA were intersected to find the potential targets of quercetin against KOA. The protein–protein interaction network was constructed through the STRING database, and the core targets were screened. Gene ontology (GO) functions enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using DAVID database. The drug–target–pathway–disease network was constructed by Cytoscape software, and the molecular docking verification was performed by Vina.

Results There were 49 potential targets for quercetin against KOA, including 10 core targets. GO functions enrichment analysis showed that the biological process of quercetin against KOA mainly involved the negative regulation of apoptotic process, collagen catabolic process, and extracellular matrix disassembly. KEGG pathway enrichment analysis showed that quercetin against KOA was closely related to PI3K-Akt signaling pathway, Rap 1 signaling pathway, FoxO signaling pathway, Ras signaling pathway, TNF signaling pathway, and ErbB signaling pathway. The results of molecular docking showed that the binding energies between ligand and receptors were less than -5 kcal • mol^{-1}.

Conclusions The molecular mechanism of quercetin against KOA involves many targets and pathways, which can regulate the proliferation and apoptosis of chondrocytes, degradation of extracellular matrix, and inflammatory reaction. Quercetin can stably bind to the active pockets of core target proteins, thereby exerting the effect against KOA.

Keywords
- quercetin
- knee osteoarthritis
- potential targets
- molecular mechanism
- network pharmacology
- molecular docking

Introduction

Knee osteoarthritis (KOA) is a chronic osteoarthropathy, which is characterized by degeneration of knee cartilage, secondary osteophyte formation, subchondral bone remodeling and synovitis.[1] Its clinical manifestations are mainly pain, swelling, functional limitation, and joint deformity around the knee, which seriously affect the health and quality of life of patients.[2]

received
October 23, 2021
accepted after revision
January 23, 2022

DOI https://doi.org/10.1055/s-0042-1747918.
ISSN 2096-918X.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

According to the theory of traditional Chinese medicine, KOA is the functional deficiency of liver and kidney, which involves vessel blocking and joint pain. Therefore, it is believed that the multidrug and multitarget approaches should be considered in the treatment of KOA. Epidemiological studies have shown that KOA is more common in middle-aged and elderly people, with 250 million people worldwide.[3] With the extension of life expectancy and the growth of the elderly population, it is expected that this number will increase gradually in the future. The occurrence of KOA may be related to many risk factors, such as age, sex, obesity, etc.,[4] and the specific pathogenesis is still unclear. Therefore, how to control KOA more effectively and delay its development process is also a hot and difficult problem in current research.

At present, most guidelines recommend non-steroidal anti-inflammatory drugs as first-line drugs, aiming at relieving pain and improving clinical symptoms in patients with KOA.[5] However, gastrointestinal symptoms (such as gastrointestinal bleeding), cardiovascular diseases (such as myocardial infarction), and other adverse reactions cannot be ignored, which limits the clinical application. KOA can be treated through various approaches such as oral administration of herbal medicine, topical treatment, physiotherapy, and so on. Quercetin, a plant-derived flavonoid that is widely found in vegetables and fruits, has multiple biological activities and pharmacological effects such as antioxidative stress, anti-inflammatory, and immune regulation.[6] Studies have shown that quercetin could reduce oxidative stress and inhibit the degradation of cartilage extracellular matrix; up-regulate SOD and TIMP-1; down-regulate MMP-13 in serum, synovial fluid, and synovial tissue; and improve the degradation of KOA.[7] Studies have shown that the mechanism of quercetin against KOA may be related to the inhibition of TLR-4/NF-κB signaling and the decrease of IL-1β and TNF-α levels.[8] It also suggested that quercetin could inhibit the apoptosis of chondrocytes, regulate the polarization of synovial macrophages to M2 macrophages, create chondrogenic environment for chondrocytes, and enhance cartilage repair in KOA environment, thus playing a protective role in cartilage.[9] Studies have shown that quercetin may be a potential candidate drug against KOA. However, the potential targets and molecular mechanisms of quercetin against KOA have not been systematically explained in detail.

Network pharmacology explains the occurrence and development of diseases from the perspective of system biology and biological network balance and understands the interaction between drugs and organisms from the overall perspective of improving or restoring the balance of biological network.[10] By constructing a multi-level network of drug, target, pathway, and disease, the potential molecular mechanism of drug treatment for diseases is predicted systematically. Molecular docking is a research method that simulates the geometric and energy matching between small drug molecules and target proteins, studies the interaction between ligands and receptors, and predicts their binding mode and affinity.[11,12] Based on network pharmacology and molecular docking methods, this study will preliminarily clarify the potential targets, signaling pathways, and molecular mechanisms of quercetin against KOA, and provide some reference and theoretical basis for future research. The flow chart of network pharmacology and molecular docking of quercetin against KOA was drawn, as shown in ►**Fig. 1**.

Methods

Screening Chemical Structure and Potential Targets of Quercetin

The chemical structure of quercetin was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[13] and downloaded in the format of "SDF." Then, the "SDF" file was uploaded to the Swiss Target Prediction database (http://www.swisstargetprediction.ch/)[14] to predict the targets of quercetin, and the limited possibility was more than 0. The corresponding targets were supplemented in the DrugBank database (https://go.drugbank.com/).[15] At last, the UniProt database (https://www.uniprot.org/)[16] was used to standardize the targets' names after removing duplicates.

Screening Disease Targets of KOA

The disease targets of KOA were collected in DisGeNET (https://www.disgenet.org/),[17] OMIM (https://omim.org/),[18] and GeneCards database (https://www.genecards.org)[19] with the keywords of "Knee osteoarthritis," "Osteoarthritis, Knee," "Osteoarthritis of Knee," and "KOA." The targets obtained in the three databases were merged, and duplicates were removed.

Screening Drug–Disease Intersection Targets

To clarify the interaction between drug targets and disease targets, the targets of both were intersected. Venn diagram of drug and disease was drawn by online tools (http://bioinformatics.psb.ugent.be/webtools/Venn/) to obtain the intersection targets of quercetin against KOA.

Constructing Protein–Protein Interaction Network and Screening Core Targets

The intersection targets were uploaded to the STRING database (https://string-db.org/),[20] the specie was set as "Homo sapiens," the minimum required interaction score was 0.4, and the disconnected nodes in the network were hidden. The node information was downloaded in the format of "TSV" and imported into Cytoscape 3.8.2 software to construct a protein–protein interaction (PPI) network. The core targets of quercetin against KOA were obtained by using cytoHubba plug-in, which was used for molecular docking.

GO and KEGG Enrichment Analysis

Gene ontology (GO) functions enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using DAVID database (https://david.ncifcrf.gov/)[21] with p-value less than 0.05, and the specie was set as "Homo sapiens." The biological process (BP), cellular component (CC), and molecular function (MF) of the top 10 GO enrichment and the signaling pathways of the top 20 KEGG enrichment were analyzed by bioinformatics platform (http://www.bioinformatics.com.cn/).

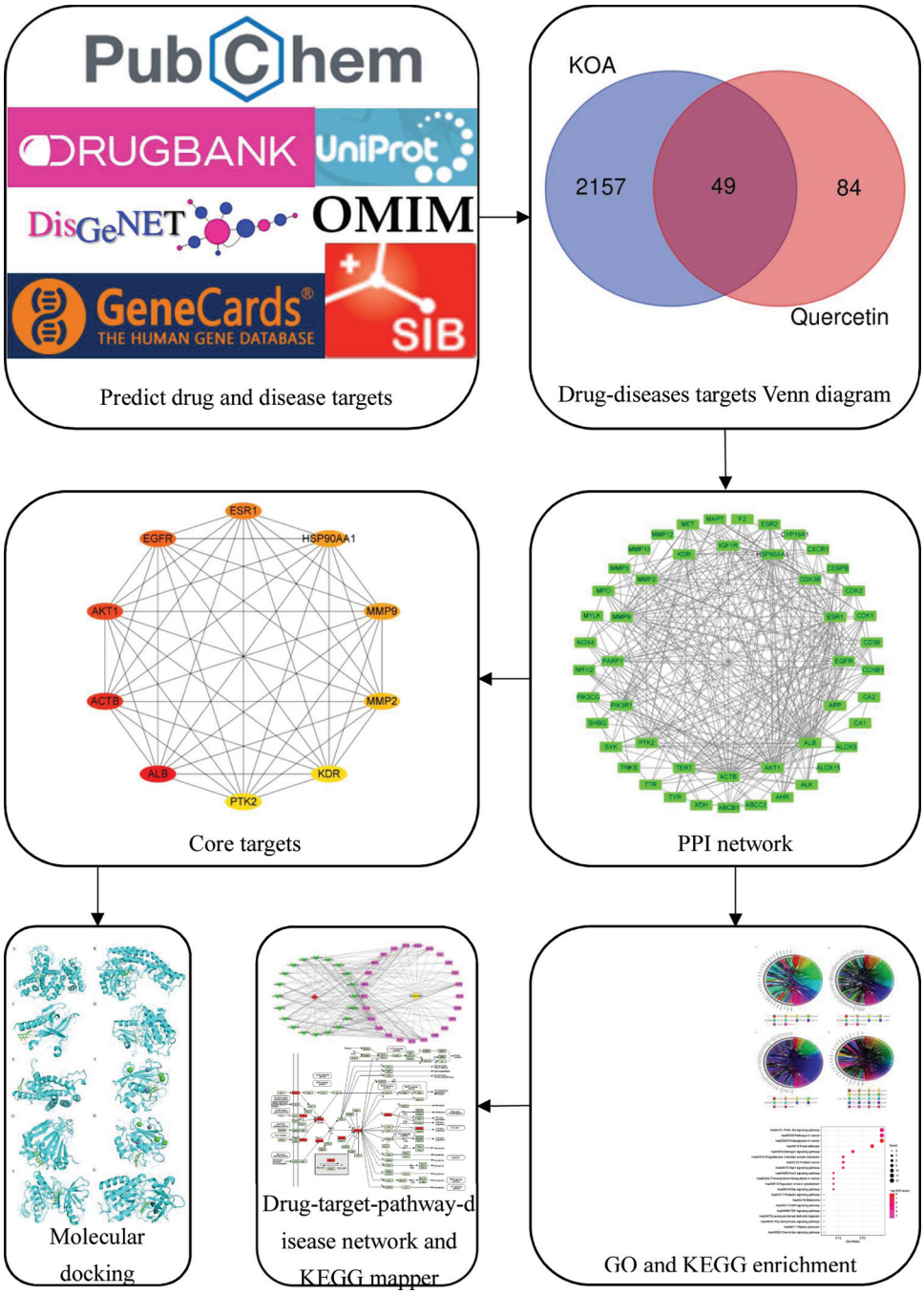


Fig. 1 Flow chart of network pharmacology and molecular docking.

Constructing Drug–Target–Pathway–Disease Network

Cytoscape 3.8.2 software was used to construct the drug–target–pathway–disease network. Nodes represented drug, target, pathway, and disease, while edges represented the interaction among them.

Molecular Docking

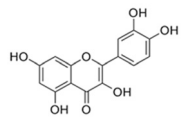
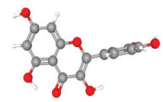
The structures of ligand and protein receptors were downloaded from PubChen dabases (https://pubchem.ncbi.nlm.nih.gov/) and PDB databases (https://www.rcsb.org/).[22] PyMol 2.4.0 software[23] was used to remove water and small molecular ligands, and then AutoDockTools 1.5.6 software was used to hydrogenate and find protein active pockets. Finally, Vina was run for molecular docking to verify the interaction between quercetin and core targets.[24]

Results

Chemical Structure and Potential Targets of Quercetin

The PubChem CID, molecular formula, and chemical structure of quercetin were obtained from PubChem database, as shown in ►**Table 1**. There were 100 targets in Swiss Target Prediction database and 40 targets in DrugBank database. After eliminating duplicate targets, 133 drug targets of quercetin were obtained.

Table 1 Chemical information of quercetin

PubChem CID	Molecular formula	2D Structure	3D Conformer
5280343	$C_{15}H_{10}O_7$		

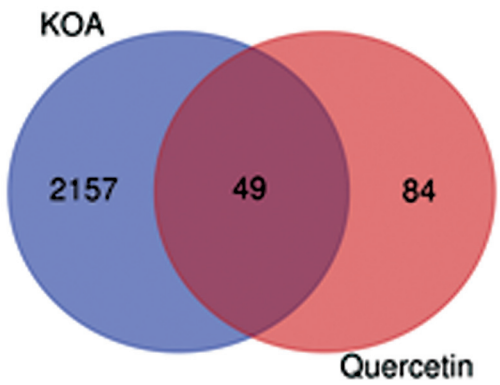


Fig. 2 Drug-disease targets Venn diagram.

Disease Targets of KOA

Searching the databases of DisGeNET, OMIM, and GeneCards, 368 targets, 30 targets, and 2 084 targets were obtained, respectively. After removing duplicate targets, 2,206 disease targets of KOA were obtained.

Drug–Disease Intersection Targets

There were 49 intersection targets for quercetin and KOA, as shown in ►**Fig. 2**.

PPI Network and Core Targets

PPI network of quercetin against KOA was constructed by Cytoscape 3.8.2 software, as shown in ►**Fig. 3**. There were 49 nodes and 319 edges in the figure. Nodes represented proteins, while edges represented the interaction between them. Ten core targets, ALB, ACTB, AKT1, EGFR, ESR1, MMP9, HSP90AA1, MMP2, PTK2, and kinase insert domain receptor (KDR) were screened out by cytoHubba plug-in.

GO and KEGG Enrichment Analysis

The targets of quercetin against KOA were enriched to 159 GO items ($p < 0.05$), including 98 BP, 22 CC pathways, and 39 MF. The top 10 items of BP, CC, and MF were selected to draw the bar graph, as shown in ►**Fig. 4**. These BP mainly involved the negative regulation of apoptotic process, collagen catabolic process, and extracellular matrix disassembly. CC mainly involved extracellular space, plasma membrane, and extracellular region. MF involved protein tyrosine kinase activity, nitric-oxide synthase regulator activity, and protein binding. At the same time, 59 signaling pathways were obtained by KEGG enrichment analysis; the bubble diagram of the top 20 signaling pathways is shown in ►**Fig. 5**. The signaling pathways of quercetin against KOA mainly involved PI3K-Akt signaling pathway,

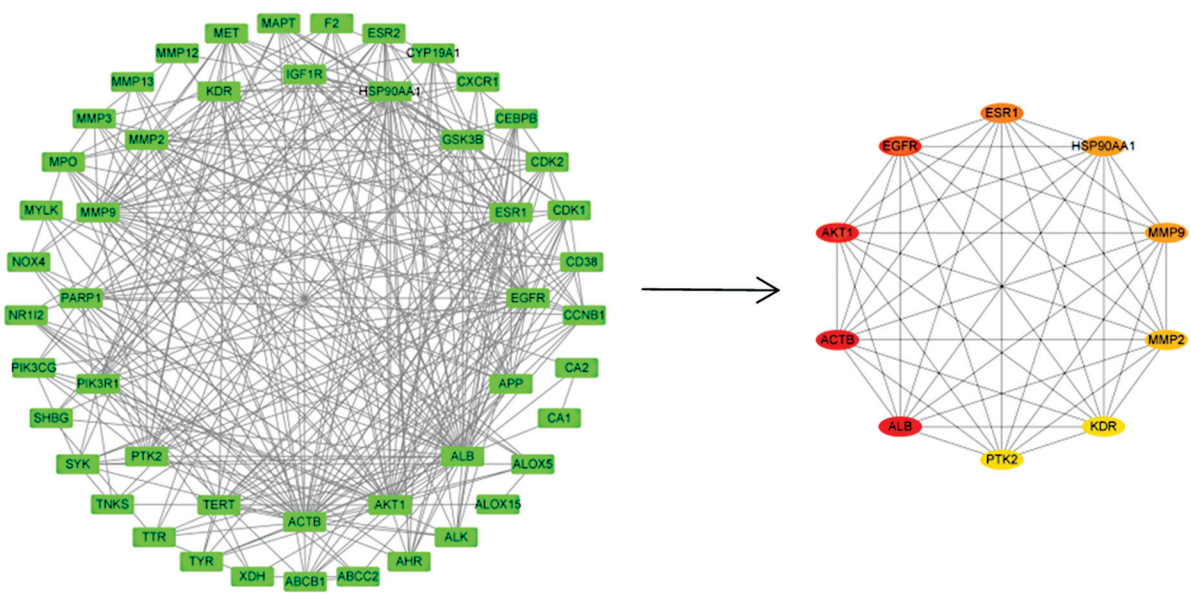


Fig. 3 PPI network and core targets.

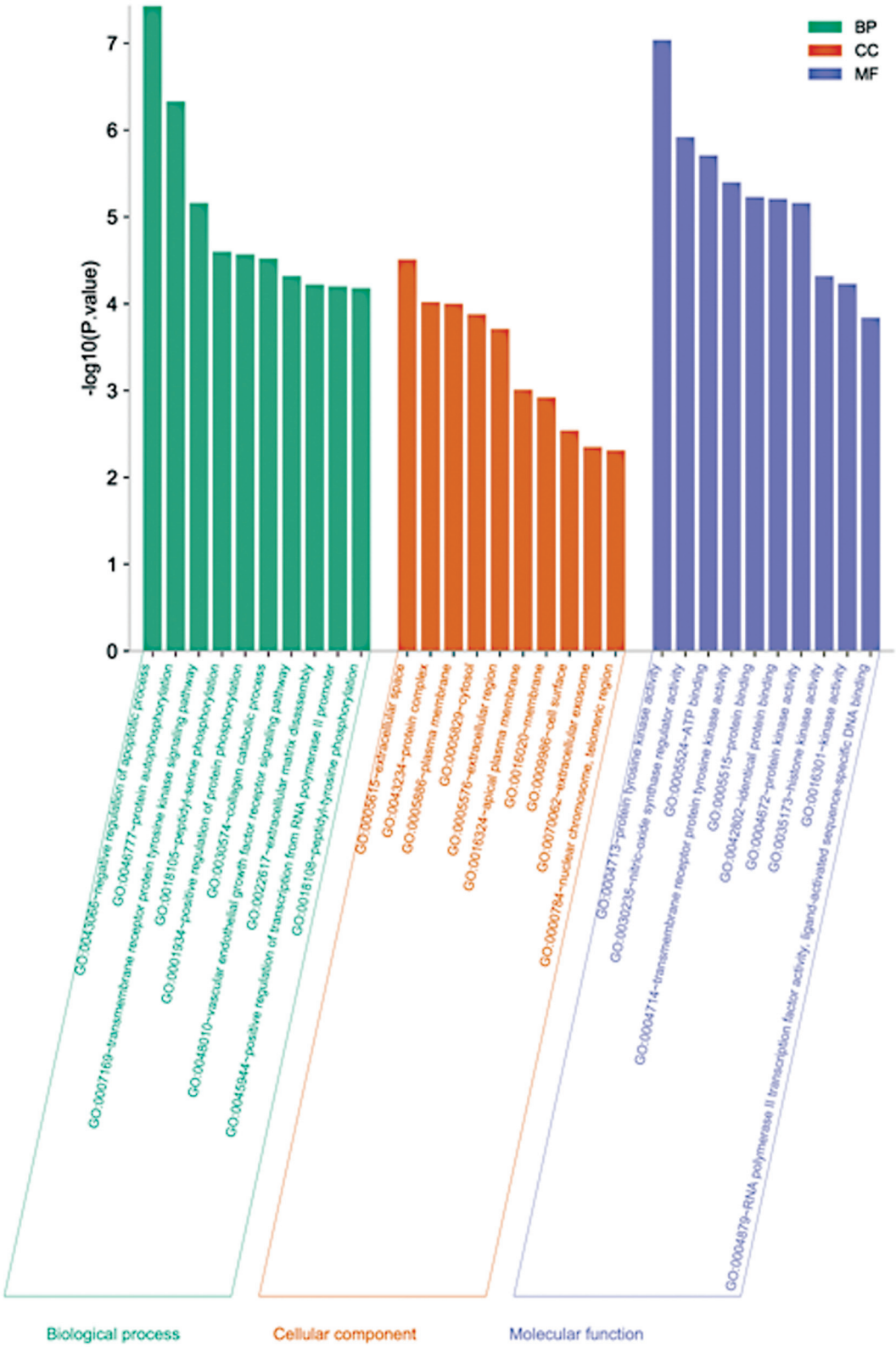


Fig. 4 GO functional enrichment analysis.

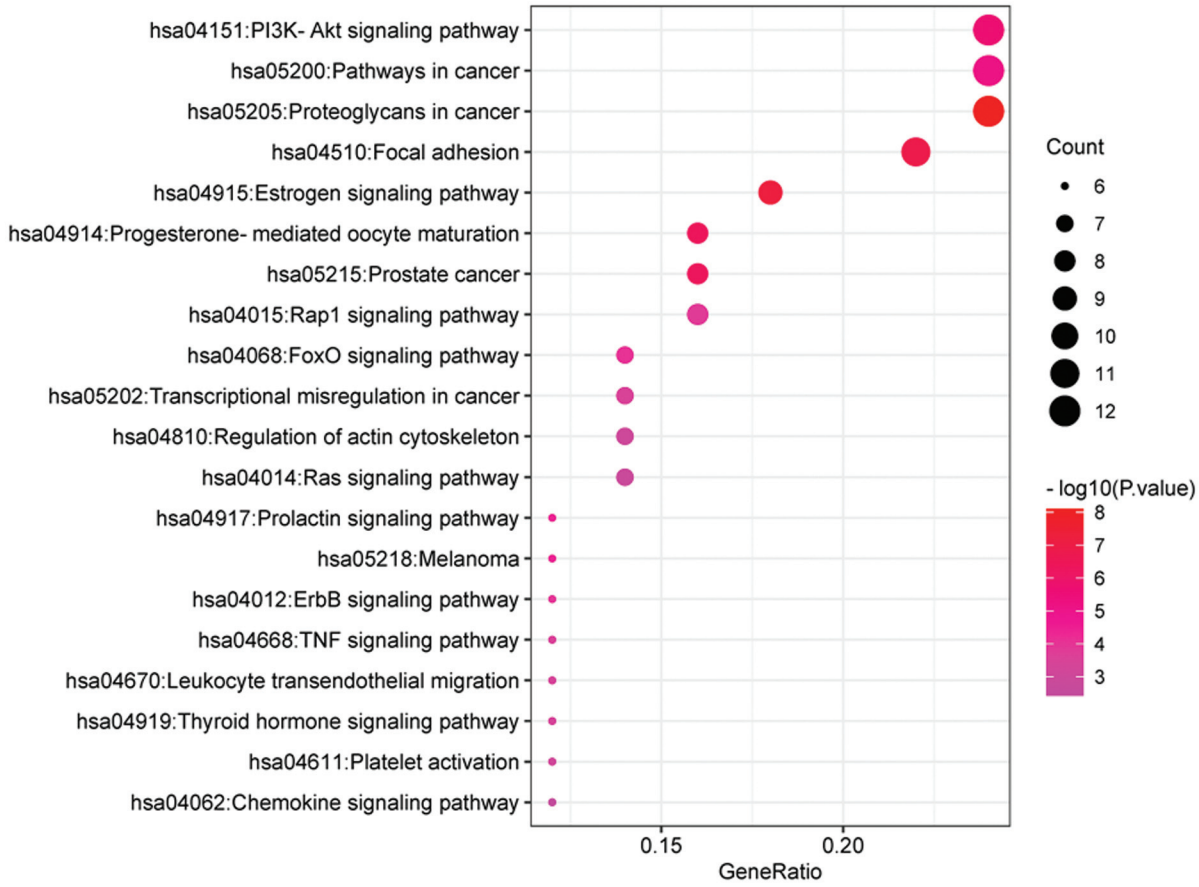


Fig. 5 KEGG pathway enrichment analysis.

Rap 1 signaling pathway, FoxO signaling pathway, Ras signaling pathway, TNF signaling pathway, ErbB signaling pathway, and so on. GO and KEGG chord diagrams (►**Fig. 6**) showed the genes enrichment of each item. Then, the important PI3K-Akt signaling pathway was drawn, as shown in ►**Fig. 7**, in which red represents the targets of quercetin against KOA enriched in this pathway.

Drug–Target–Pathway–Disease Network

The drug–target–pathway–disease network was constructed by Cytoscape 3.8.2 software, as shown in ►**Fig. 8**. In the figure, yellow ellipses represent quercetin, red diamond represents KOA, purple square represents targets, green triangle represents signaling pathways, and gray line represents the interaction among them.

Molecular Docking

The core targets were docked with quercetin in turn, as shown in ►**Fig. 9**. The binding activity of the two could be evaluated according to the binding energy. If the value was less than 0, it meant that they could spontaneously combine. The lower the binding energy, the easier it was to bind. Generally, it was considered that binding energy less than -5.00 kcal • mol^{-1} meant good binding activity. The binding energy of molecular docking is shown in ►**Table 2**, with an average of -8.47 kcal • mol^{-1}.

Discussion

KOA is a common and frequently occurring disease among middle-aged and elderly people. With the extension of life expectancy and the arrival of an aging society, the morbidity and disability rate are gradually increasing. Epidemiological studies showed that the prevalence rate of symptomatic KOA among middle-aged and elderly people in China was 8.1%,[25] while the incidence rate in rural areas was as high as 16.57% and the incidence rate of elderly people over 70 years old was obviously increasing (29.25% for women and 24.71% for men).[26] The World Health Organization pointed out in *The Global Burden of Disease* that KOA has become the fourth largest disabling disease in the world,[27] which has brought more and more patients physical and mental pain and torture and brought great mental pressure and heavy economic burden to many families and societies.[28] KOA, as a chronic degenerative disease, will cause joint injury and irreversible disability if it is not treated properly. However, at present, there is no cure for its recurrence, and drug treatment has reached a certain bottleneck.

In recent years, quercetin has gradually been used in the treatment of KOA. However, the potential targets, signaling pathways, and molecular mechanisms of quercetin against KOA still need further study. The pathophysiological mechanism and complexity of KOA are generally considered to

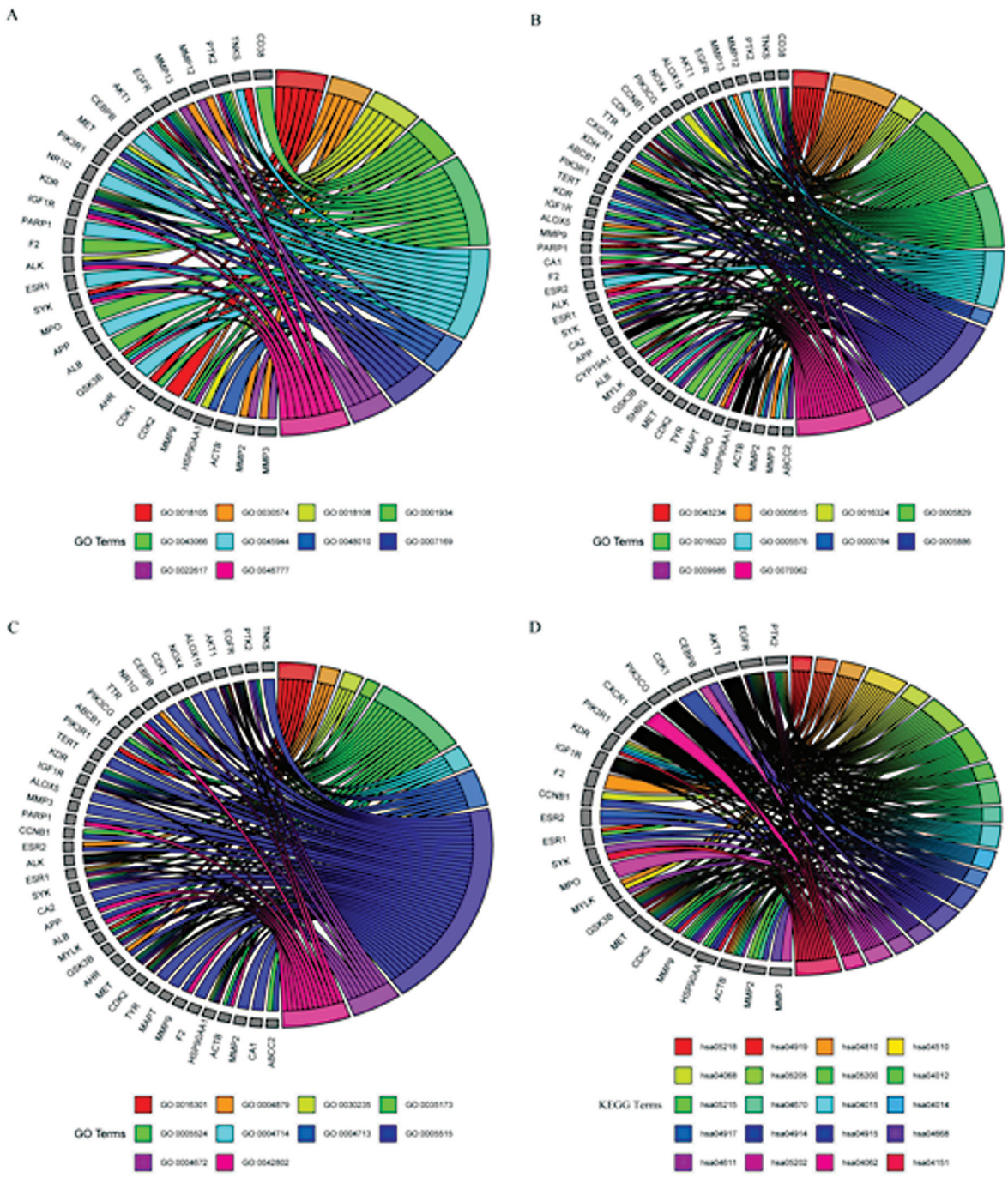


Fig. 6 *GO and KEGG chord diagrams.* (**A**) BP chord diagram; (**B**) CC chord diagram; (**C**) MF chord diagram; and (**D**) KEGG chord diagram.

be a variety of BP and multiple signaling pathways involved in its damage process. In the experimental study, it is difficult to systematically and comprehensively analyze the relationship among drugs, target proteins, and signaling pathways. With the rapid development of molecular biology, bioinformatics, pharmacology, and computer science, network pharmacology explains the interaction between drugs and targets from multi-level and multi-angle interaction networks and reveals the mechanism of drugs under the theoretical framework of drug, target, and disease.[29] Therefore, this study attempts to analyze the potential targets and molecular mechanisms of quercetin against KOA from a microscopic perspective by means of network pharmacology and molecular docking, so as to provide new insights and ideas for the pathogenesis and drug treatment of KOA.

By searching the databases, 133 drug targets and 2 206 disease targets were obtained, including 49 intersection targets and 10 core targets. According to the results of molecular docking, the binding energies between ligand

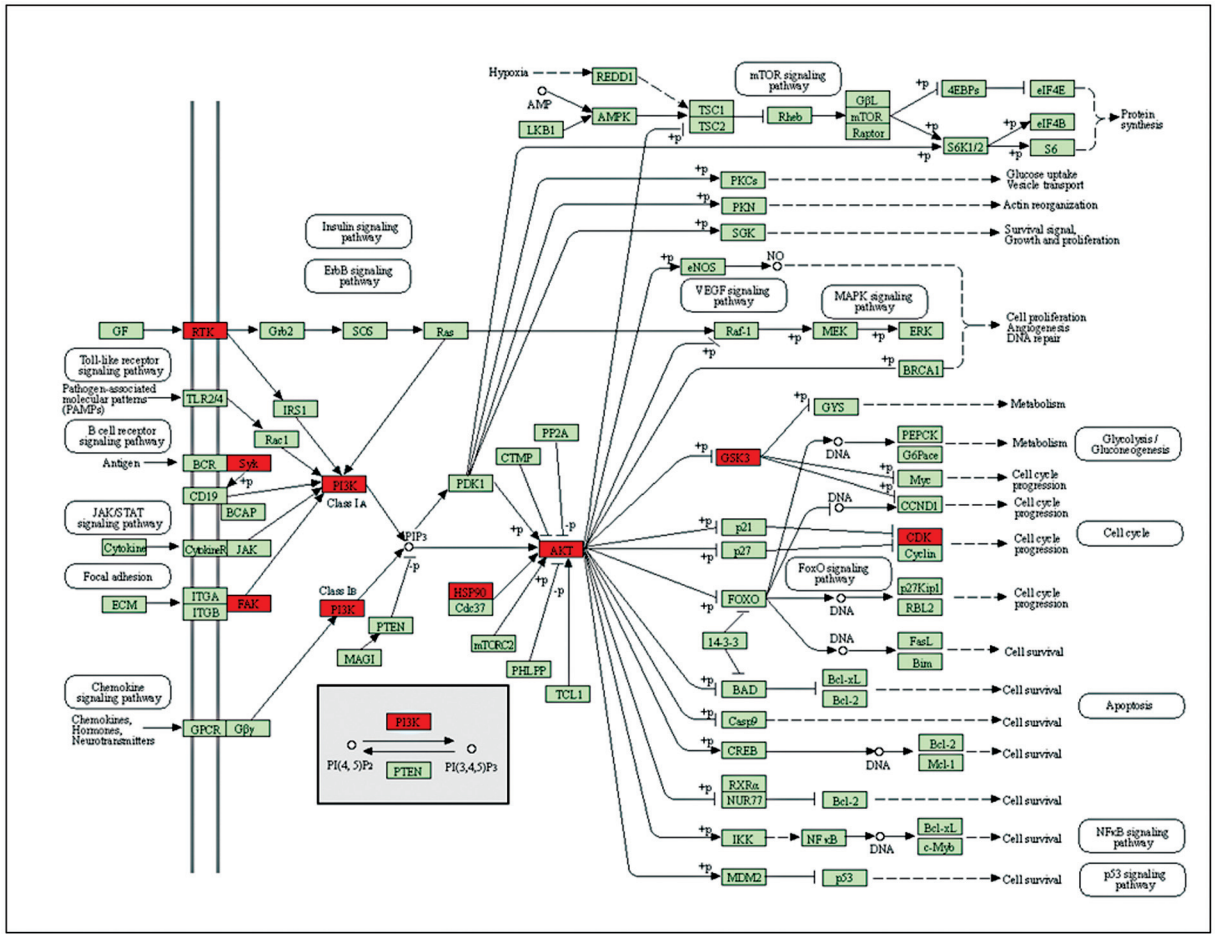


Fig. 7 PI3K-Akt signaling pathway.

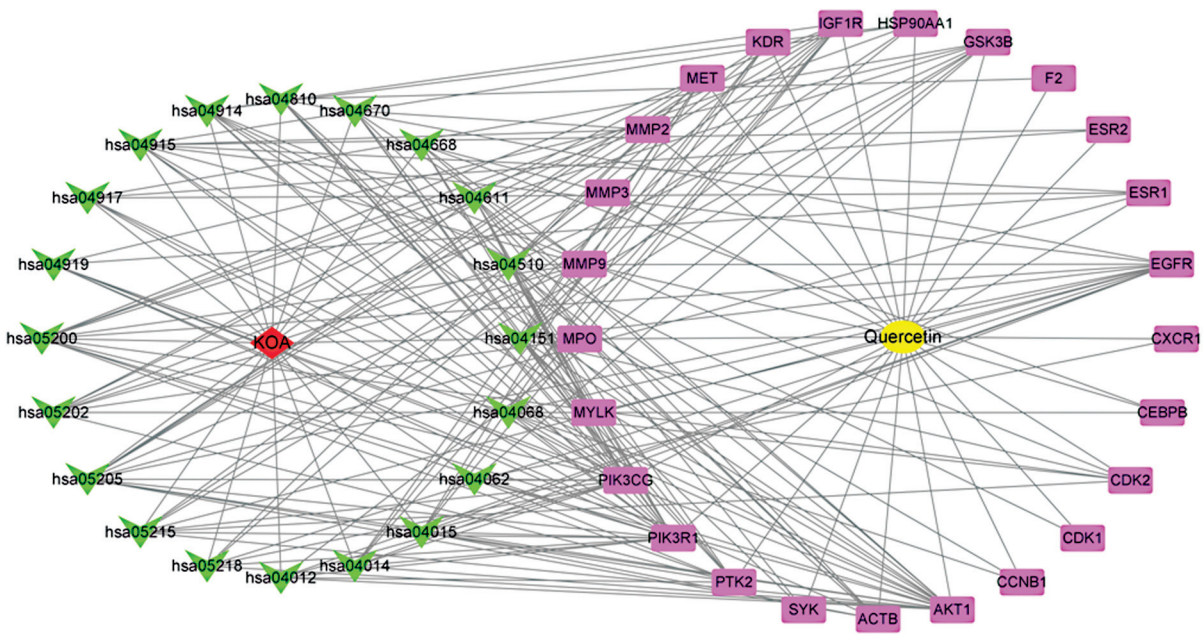


Fig. 8 Drug–target–pathway–disease network.

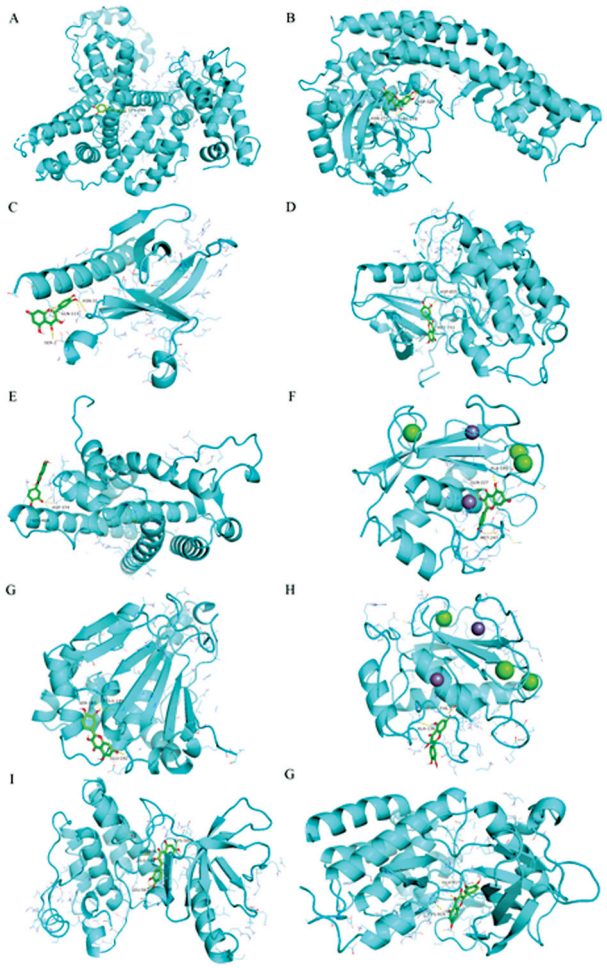


Fig. 9 *Molecular docking.* (**A**) ALB, (**B**) ACTB, (**C**) AKT1, (**D**) EGFR, (**E**) ESR1, (**F**) MMP9, (**G**) HSP90AA1, (**H**) MMP2, (**I**) PTK2, and (**J**) KDR.

and receptors were all less than −5 kcal • mol^{-1}, which indicates that quercetin can stably bind receptor protein and exert a therapeutic effect. The binding energy between quercetin and MMP9 was the lowest, and the docking result was the best. Quercetin can stably bind to the active pocket of MMP-9 protein structure through hydrogen bond interaction among amino acid residues ALA-189, GLN-227, and MEF-247. Second, ACTB, KDR, PTK 2, MMP 2, and EGFR also have lower binding energy, which are important core targets of quercetin against KOA.

MMP is a family of zinc-dependent proteolytic enzyme, which mainly participates in the degradation of extracellular matrix by decomposing collagen and proteoglycan and plays an important role in the pathological process of KOA articular cartilage matrix and the damage of chondrocytes.[30] Under normal circumstances, the synthesis and degradation of articular chondrocytes and extracellular matrix are in a dynamic equilibrium. When KOA occurs, this state is broken, extracellular matrix is degraded, and chondrocytes are induced to oxidize under the stressed cell environment, and finally, chondrocyte apoptosis is induced. Gelatinases, including gelatinase A (MMP-2) and gelatinase B (MMP-9), are the major contributors to the degradation of extracellular matrix.[31] It is well known that MMP-2 is produced by stromal cells in the subsynovial layer, while MMP-9 is secreted by neutrophils, macrophages, and synovial cells. Research works have shown that the expression of these two matrix metalloproteinases is stronger in superficial chondrocytes, and the degeneration is more obvious in superficial chondrocyte.[32] *ACTB* is a housekeeping gene of KOA synovial membrane gene expression research,[33] which is relatively conservative and mainly involved in cell movement and contraction. VEGF receptor 2, also known as KDR, has anti-apoptotic effect after activation, which is mediated by PI3K-Akt signaling pathway.[34] VEGF can also induce the production of pro-inflammatory cytokines IL-6, CXC chemokine IL-8, and MMP-13.[35] In addition to promoting angiogenesis, VEGF also has a strong pro-inflammatory effect.[36] Focal adhesion kinase (FAK) encoded by PTK is a non-receptor protein tyrosine kinase. By inhibiting abnormal bone formation induced by H-type vessels and specifically inhibiting FAK signaling in subchondral bone, subchondral bone degeneration and articular cartilage degeneration can be alleviated.[37] EGFR is an important regulator of cartilage matrix degradation during cartilage development, which can delay the progress of KOA by down-regulating the survival of chondrocytes and the degradation of extracellular matrix.[38]

To further understand the BP and signaling pathways of quercetin against KOA, GO functional enrichment analysis and KEGG pathway enrichment analysis of intersection targets were performed. GO enrichment analysis showed that the BP of quercetin against KOA mainly involved the negative regulation of apoptotic process, collagen catabolic process, and extracellular matrix disassembly, which were undoubtedly closely related to the occurrence and development of KOA. KEGG analysis showed that apoptosis, oxidative stress, and inflammatory reaction enriched more target genes, and PI3K-Akt signaling pathway was more important.

PI3K-Akt signaling pathway is a classical anti-apoptosis pathway, which is composed of intracellular phosphatidylinositol kinase PI3K and important downstream target Akt. Akt is a serine/threonine protein kinase, which is activated by extracellular factors through PI3K-dependent phosphorylation.[39] After phosphorylation of Akt, it can inhibit the activity of downstream pro-apoptotic protein Bax and

Table 2 The binding energy of molecular docking (−kcal • mol^{-1})

ALB	ACTB	AKT1	EGFR	ESR1	MMP9	HSP90AA1	MMP2	PTK2	KDR
−8.2	−9.9	−6.4	−8.3	−7.2	−10.7	−6.4	−8.7	−9.1	−9.8

promote the activation of anti-apoptotic protein Bcl-2, thus promoting chondrocyte proliferation, inhibiting chondrocyte apoptosis, and slowing down the process of KOA cartilage degeneration.[40] FoxO is a transcription factor inducing apoptosis. With the increase of age, the expression of FoxO on cartilage surface decreases obviously, which promotes the release of inflammatory mediators and induces cartilage degradation. The function of FoxO in cartilage growth is mainly mediated by FoxO1, which is closely related to proliferation, survival, and differentiation of chondrocytes.[41] When FoxO is phosphorylated by Akt, it will cause FoxO1 to transfer from the nucleus to the cytoplasm and lose its transcriptional activity, which will help the survival, growth, and proliferation of cells and avoid apoptosis. In addition, FoxO signaling pathway is especially important in regulating oxidative stress. It was found that the down-regulation of FoxO transcription factor in chondrocytes decreased the activity of cells against oxidative stress, which may be related to the decrease of antioxidant proteins and autophagy-related proteins.[42] That is to say, chondrocytes with decreased expression of FoxO transcription factor are more likely to die under oxidative stress.

However, PI3K-Akt signaling pathway is closely related to autophagy, inflammation, and metabolism of extracellular matrix in KOA pathology.[40] Specifically, activated Akt can directly activate mTOR, which is an important downstream signaling molecule that regulates autophagy. Studies have found that inhibiting the PI3K-Akt-mTOR pathway increased the autophagy of rat articular chondrocytes and alleviated the inflammatory response of arthritis rats.[43] In addition, PI3K-Akt signaling pathway can also act synergistically with the downstream protein NF-κB. Akt activates IκB kinase (IKKα), which leads to the degradation of IκB, an inhibitor of NF-κB, and the transfer of NF-κB to nucleus. It is found that NF-κB can lead to the production of matrix-degrading enzymes, proinflammatory cytokines, and inflammatory mediators by coordinating multi-layer signal networks, thus accelerating the occurrence and development of diseases.[44] In other words, simply inhibiting or activating PI3K-Akt signaling pathway will affect the occurrence and development of KOA. Ras protein and Rap1 protein belong to the small molecule G protein Ras superfamily. When combined with GTP, they are activated, and when combined with GDP, they are inactivated. They are very important to control the proliferation, differentiation, apoptosis, and survival of cells.[45] Once Ras protein is activated, many signal transduction pathways will be activated, such as MAPK and PI3K.[34] It is well known that PI3K can be directly activated by receptor tyrosine kinase or G protein-coupled receptor, which makes the Ras signal network very complex.[46] ErbB is a tyrosine kinase receptor. When it binds to the ligand, it activates PI3K-Akt, a downstream signaling pathway. The research shows that inhibition or inactivation of ErbB2 receptor leads to up-regulation of Bax expression and down-regulation of Bcl-2 expression.[47] TNF signaling pathway can mediate KOA inflammatory reaction, promote the production of inflammatory cytokines, and lead to synovium hyperplasia and inflammation. In addition, quercetin can regulate chemokines, thus affecting the chemotaxis of leukocytes to inflammatory sites. Therefore, there may be complex interactions between these pathways, which cooperatively regulate the proliferation and apoptosis of chondrocytes, degradation of extracellular matrix, and inflammatory reaction.

There are still some shortcomings in this research. First of all, there may be some problems in the database itself, such as incomplete drug targets and disease targets, low accuracy, and possible deviations in the results. Second, the research result is a virtual prediction result. Although a large number of targets and pathways can be screened out, only molecular docking verification is performed and experimental verification is not added. Therefore, our future research will further verify the mechanism of quercetin against KOA in combination with in vitro and in vivo experiments.

Conclusion

To sum up, a total of 133 drug targets and 2 206 disease targets were obtained, including 49 intersection targets and 10 core targets. Quercetin may directly or indirectly regulate PI3K-Akt signaling pathway, Rap1 signaling pathway, FoxO signaling pathway, Ras signaling pathway, TNF signaling pathway, ErbB signaling pathway, and chemokine signaling pathways by regulating the expression of MMP9, ACTB, KDR, PTK3, MMP2, and EGFR protein targets, so as to play a role in alleviating chondrocyte apoptosis, reducing immune-inflammatory response, improving human antioxidant stress response, and strengthening chondrocyte proliferation. Therefore, the effect of quercetin against KOA is related to the coordinated regulation of multiple targets, multiple BP, and signaling pathways. This study can provide a reference and theoretical basis for subsequent experiments in vitro and in vivo.

Credit Authorship Contribution Statement

Lingling Li: Conceptualization, writing-original draft. **Hailiang Huang:** Methodology, supervision, and writing - review & editing.

Funding

This work was funded by the Project of Chinese Medicine Science and Technology Development Planning in Shandong Province (2017-018), the Project of Scientific Research and Development Plan of Colleges and Universities in Shandong Province (J18KB130), and the Project of the First Batch of Excellent Scientific Research and Innovation Team in Shandong University of Chinese Medicine (220316).

Conflict of Interest

The authors declare no conflict of interest.

References

1 Hügle T, Geurts J. What drives osteoarthritis?Synovial versus subchondral bone pathology Rheumatology (Oxford) 2017;56 (09):1461–1471

2 Madry H, Kon E, Condello V, et al. Early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc 2016;24(06):1753–1762

3 Lee BJ, Yang S, Kwon S, Choi KH, Kim W. Association between metabolic syndrome and knee osteoarthritis: a cross-sectional nationwide survey study. J Rehabil Med 2019;51(06):464–470
4 Roos EM, Arden NK. Strategies for the prevention of knee osteoarthritis. Nat Rev Rheumatol 2016;12(02):92–101
5 Kolasinski SL, Neogi T, Hochberg MC, et al. 2019 American college of rheumatology/arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. Arthritis Care Res (Hoboken) 2020;72(02):149–162
6 Basu A, Schell J, Scofield RH. Dietary fruits and arthritis. Food Funct 2018;9(01):70–77
7 Wei B, Zhang Y, Tang L, Ji Y, Yan C, Zhang X. Protective effects of quercetin against inflammation and oxidative stress in a rabbit model of knee osteoarthritis. Drug Dev Res 2019;80(03): 360–367
8 Zhang J, Yin J, Zhao D, et al. Therapeutic effect and mechanism of action of quercetin in a rat model of osteoarthritis. J Int Med Res 2020;48(03):300060519873461
9 Hu Y, Gui Z, Zhou Y, Xia L, Lin K, Xu Y. Quercetin alleviates rat osteoarthritis by inhibiting inflammation and apoptosis of chondrocytes, modulating synovial macrophages polarization to M2 macrophages. Free Radic Biol Med 2019;145:146–160
10 Luo TT, Lu Y, Yan SK, Xiao X, Rong XL, Guo J. Network pharmacology in research of Chinese medicine formula: methodology, application and prospective. Chin J Integr Med 2020;26(01): 72–80
11 Vakser IA. Protein-protein docking: from interaction to interactome. Biophys J 2014;107(08):1785–1793
12 Xue LC, Dobbs D, Bonvin AM, Honavar V. Computational prediction of protein interfaces: a review of data driven methods. FEBS Lett 2015;589(23):3516–3526
13 Kim S, Chen J, Cheng T, et al. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Res 2021;49(D1): D1388–D1395
14 Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res 2019;47(W1):W357–W364
15 Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 2018;46(D1):D1074–D1082
16 Uniprot CUniProt Consortium. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 2019;47(D1):D506–D515
17 Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, et al. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res 2020;48(D1):D845–D855
18 Amberger JS, Bocchini CA, Scott AF, Hamosh A. OMIM.org: leveraging knowledge across phenotype-gene relationships. Nucleic Acids Res 2019;47(D1):D1038–D1043
19 Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics 2016;54:1, 33
20 Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47(D1):D607–D613
21 Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4(01):44–57
22 Burley SK, Bhikadiya C, Bi C, et al. RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Res 2021;49(D1):D437–D451
23 Mooers BHM. Shortcuts for faster image creation in PyMOL. Protein Sci 2020;29(01):268–276
24 El-Hachem N, Haibe-Kains B, Khalil A, Kobeissy FH, Nemer G. AutoDock and AutoDockTools for protein-ligand docking: beta-site amyloid precursor protein cleaving enzyme 1(BACE1) as a case study. Methods Mol Biol 2017;1598:391–403
25 Tang X, Wang S, Zhan S, et al. The prevalence of symptomatic knee osteoarthritis in China: results from the China health and retirement longitudinal study. Arthritis Rheumatol 2016;68(03): 648–653
26 Liu Y, Zhang H, Liang N, et al. Prevalence and associated factors of knee osteoarthritis in a rural Chinese adult population: an epidemiological survey. BMC Public Health 2016;16:94
27 Cross M, Smith E, Hoy D, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73(07):1323–1330
28 Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. Nat Rev Rheumatol 2014;10(07): 437–441
29 Zhang W, Bai Y, Wang Y, Xiao W. Polypharmacology in drug discovery: a review from systems pharmacology perspective. Curr Pharm Des 2016;22(21):3171–3181
30 Rose BJ, Kooyman DL. A tale of two joints: the role of matrix metalloproteases in cartilage biology. Dis Markers 2016; 2016:4895050
31 Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci 2017; 147:1–73
32 Lipari L, Gerbino A. Expression of gelatinases (MMP-2, MMP-9) in human articular cartilage. Int J Immunopathol Pharmacol 2013; 26(03):817–823
33 Watanabe H, Ishii H, Takahashi K, Takai S, Ozawa H. Suitable reference gene selection for gene expression studies in knee osteoarthritis synovium using quantitative PCR analysis. Connect Tissue Res 2018;59(04):356–368
34 Cuesta C, Arévalo-Alameda C, Castellano E. The importance of being PI3K in the RAS signaling network. Genes (Basel) 2021;12 (07):1094
35 Hao Q, Wang L, Tang H. Vascular endothelial growth factor induces protein kinase D-dependent production of proinflammatory cytokines in endothelial cells. Am J Physiol Cell Physiol 2009; 296(04):C821–C827
36 Pufe T, Harde V, Petersen W, Goldring MB, Tillmann B, Mentlein R. Vascular endothelial growth factor (VEGF) induces matrix metalloproteinase expression in immortalized chondrocytes. J Pathol 2004;202(03):367–374
37 Wu H, Xu T, Chen Z, et al. Specific inhibition of FAK signaling attenuates subchondral bone deterioration and articular cartilage degeneration during osteoarthritis pathogenesis. J Cell Physiol 2020;235(11):8653–8666
38 Zhang X, Zhu J, Liu F, et al. Reduced EGFR signaling enhances cartilage destruction in a mouse osteoarthritis model. Bone Res 2014;2:14015
39 Ke H, Mou X, Xia Q. Remifentanil repairs cartilage damage and reduces the degradation of cartilage matrix in post-traumatic osteoarthritis, and inhibits IL-1β-induced apoptosis of articular chondrocytes via inhibition of PI3K/AKT/NF-κB phosphorylation. Ann Transl Med 2020;8(22):1487
40 Sun K, Luo J, Guo J, Yao X, Jing X, Guo F. The PI3K/AKT/mTOR signaling pathway in osteoarthritis: a narrative review. Osteoarthritis Cartilage 2020;28(04):400–409
41 Matsuzaki T, Alvarez-Garcia O, Mokuda S, et al. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. Sci Transl Med 2018;10(428):eaan0746
42 Akasaki Y, Alvarez-Garcia O, Saito M, Caramés B, Iwamoto Y, Lotz MK. FoxO transcription factors support oxidative stress resistance in human chondrocytes. Arthritis Rheumatol 2014;66(12):3349–3358
43 Xue JF, Shi ZM, Zou J, Li XL. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. Biomed Pharmacother 2017;89:1252–1261

44 Choi MC, Jo J, Park J, Kang HK, Park Y. NF-κB signaling pathways in osteoarthritic cartilage destruction. Cells 2019;8 (07):734
45 Looi CK, Hii LW, Ngai SC, Leong CO, Mai CW. The role of Ras-associated protein 1 (Rap1) in cancer: bad actor or good player? Biomedicines 2020;8(09):E334
46 Krygowska AA, Castellano E. PI3K: a crucial piece in the RAS signaling puzzle. Cold Spring Harb Perspect Med 2018;8(06):a031450
47 Soliman AM, Alqahtani AS, Ghorab M. Novel sulphonamide benzoquinazolinones as dual EGFR/HER2 inhibitors, apoptosis inducers and radiosensitizers. J Enzyme Inhib Med Chem 2019; 34(01):1030–1040