

Evidence-Based Complementary and Alternative Medicine Network Pharmacology and Molecular Docking-Based Analysis for Investigating the Mechanism of Quercetin in Treating Rheumatoid Arthritis

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Abstract:

Quercetin has good effects in the treatment of rheumatoid arthritis but its mechanism of action is not completely elucidated. Objective: To investigate potential targets of quercetin's effects on rheumatoid arthritis (RA) using a network pharmacology and molecular docking approach. Methods: Quercetin (Que) together with its drug targets was determined by network analysis and then molecular docking was used to verify. First, the database was mined for information related to predicted quercetin targets and RA-related targets. Second, Que-RA target gene networks were established in Cytoscape 3.9.1 software, and subsequently, the candidate targets were screened. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were implemented via the DAVID database. Meanwhile, Cytoscape 3.9.1 software was executed to construct the drug-target-pathway-disease network. Finally, quercetin was molecularly docked with the screened targets one by one. Results: Altogether, 372 drug targets and 3418 disease targets were acquired, containing 187 targets for quercetin against RA and 10 central targets. A total of 634 GO items were enriched, and 142 signaling pathways were gained through KEGG enrichment analysis. Quercetin docked with 10 core targets sequentially, with a mean binding energy of $-8.0 \text{ kcal} \cdot \text{mol}^{-1}$. Conclusion: Quercetin can participate in the modulation of PI3K-Akt, FoxO, Rap1, and Ras signaling pathways by regulating the expression of HSP90AA1, AKT1, MAPK8, MAPK14, and other protein targets, thereby reducing chondrocyte apoptosis, alleviating immune inflammatory response, improving synovial hyperplasia, and inhibiting angiogenesis.

Keywords:

Quercetin, Rheumatoid Arthritis, Medicine Network Pharmacology, Molecular Docking

1. Introduction

Rheumatoid arthritis (RA) is defined as a chronic systemic autoimmune disease [1] featured with inflammatory changes in joint synovial tissue, cartilage, and bone, with a global incidence of 0.5%-1% [2]. The morbidity of RA in women is markedly higher than that in men [3]. The clinical manifestations of early RA include joint swelling and pain, redness, morning stiffness, and even limited mobility . At present, the focus of RA treatment is mainly on the relieving of clinical symptoms [4], Despite the effectiveness of drug therapy, there are also many side effects and these drugs do not work for some patients [5], therefore, it is necessary to develop new drugs to treat RA. With the continuous exploration of traditional Chinese medicine (TCM), its role RA therapy is worthy of an in-depth study. Quercetin, a flavonoid compound that is abundant in fruits and vegetables [6], has good effects in the treatment of RA [7], but its mechanism of action is not completely elucidated. Hopkins first stated

the notion of network pharmacology [8], which is analyzed from a systems biology and biological network balance perspective to explain disease onset and progression, and drug-body interactions, and to provide direction for the discovery of novel drugs. In our paper, the network pharmacology approach was implemented to elucidate the mechanism of quercetin in RA therapy at the molecular level. The research process is as follows (Figure 1):

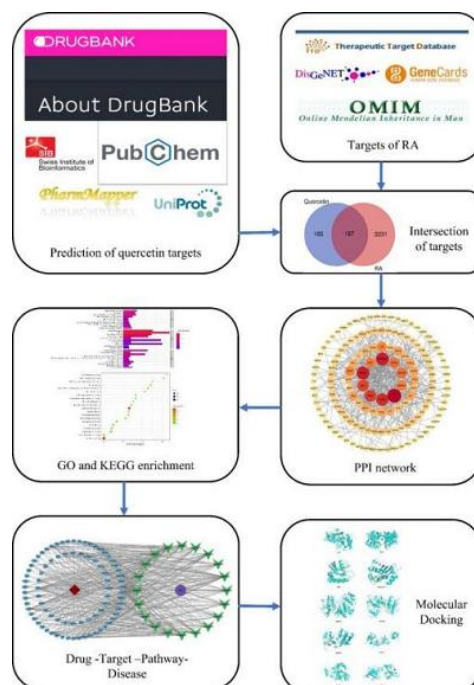


Figure 1. Schematic diagram of the research process.

2. Materials and Methods

2.1. Preparation of Data

2.1.1. Chemical Structure of Quercetin and Screening of Potential Targets

The downloading of the 2D structure of quercetin was achieved by the PubChem (<https://pubchem.ncbi.nlm.nih.gov>) database, followed by saving its sdf format and importing the 2D-sdf format into the SwissTargetPrediction (<http://swisstargetprediction.ch/>) database for the prediction of potential targets. Next, the target genes for quercetin were screened out from PharmMapper (<http://www.ilab-ecust.cn/pharmmapper/>) coupled with DrugBank (<https://go.drugbank.com/>) databases. The target gene of quercetin was obtained by merging the targets of these 3 databases, deduplicating them, and correcting the gene names in the UniProt (<https://www.uniprot.org>) database.

2.1.2. Screening of Disease Targets in RA

Four databases, including the TDD database (<https://db.idrblab.org/ttd/>), OMIM database (<http://omim.org/>), DisGeNET database (<https://www.disgenet.org/>), and GeneCards (<https://www.genecards.org/>) database were utilized to search for R related target genes. In these four databases, the keyword “rheumatoid arthritis” was utilized as a query to search for the known disease treatment targets, some genes in the GeneCards database were deleted according to “Relevance score”, and the four databases were merged and deduplicated to acquire RA-related target genes.

2.2. Screening of Cross-Targets of Drug and Disease

A Venn diagram of drug and disease was plotted with the online tool Venny2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/>) to acquire the interaction targets of quercetin and RA.

2.3. Establishment of Protein-Protein Interaction Network and Screening for the Central Targets

The protein-protein interaction (PPI) network function of the intersection targets was processed with the STRING database (<https://string-db.org/>). Meanwhile, the species were set as “homo sapiens” with a 0.9 interaction score, followed by hiding the disconnected nodes in the network. Cytoscape 3.9.1 software was used for visual analysis.

2.4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analyses

The performance of the GO and KEGG enrichment analyses were achieved by the DAVID database (<https://david.ncifcrf.gov/>) with no more than 0.05 p-value. The species were set as “Homo sapiens”. The topmost 10 GO-enriched biological processes (BP), cellular components (CC), as well as molecular functions (MF), in a combination with the topmost 20 KEGG-enriched signaling pathways, were processed.

2.5. Establishment of Drug-Target-Pathway-Disease Network

The utilization of the Cytoscape 3.9.1 software was for constructing the drug-target-pathway-disease network. In detail, nodes correspond to drugs, targets, pathways, as well as diseases, and edges refer to interactions between them.

2.6. Molecular Docking

The downloading of the ligands and protein receptors' structures were completed with PubChem (<https://pubchem.ncbi.nlm.nih.gov>) coupled with the PDB database (<https://www.rcsb.org/>). Water and small molecule ligands were discarded with

PyMol 2.5.0 software, followed by hydrogenation and search for protein active pockets with AutoDockTools 1.5.6 software. Lastly, molecular docking was implemented with Vina to validate the reciprocity between quercetin and central targets.

3. Results and Discussion

3.1. Potential Targets of Quercetin

Searching the SwissTargetPrediction database, the PharmMapper database, along with the DrugBank database yielded 103, 290, and 28 targets, respectively. Upon the removal of repeated targets, 372 drug targets of quercetin were acquired.

3.2. Disease Targets of RA

Searching the TDD, DisGeNET, OMIM, as well as GeneCards databases yielded 137, 2,722, 529, and 1174 targets, respectively. Upon the removal of duplicate targets, 3,418 disease targets of RA were acquired.

Cross-Targets of Drug and Disease

There were 187 targets for quercetin against RA (Figure 2).

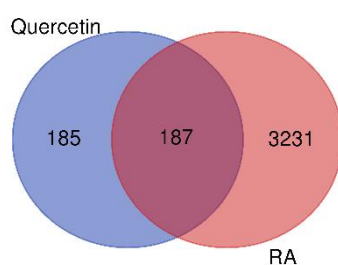


Figure 2. There were 187 targets for quercetin against RA.

3.3. PPI Network and Core Objectives

The performance of the PPI network of quercetin against RA was achieved with Cytoscape 3.9.1 software (Figure 3).

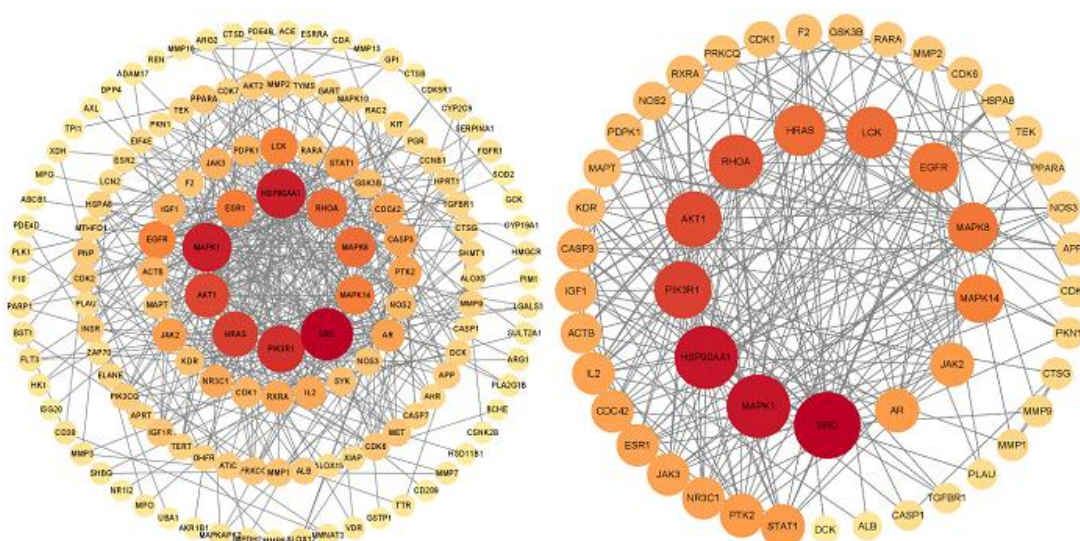


Figure 3. PPI network.

The network graph contained 141 nodes and 468 edges. The topology of the PPI network was analyzed using the “Analyze Network” function, and the degree value, betweenness centrality (BC), along with closeness centrality (CC) were calculated for each node, among which the median degree number was 4, the median of BC was 0.0017, and the median of CC was 0.2992. There were 50 matching target proteins screened following the criteria of degree ≥ 4 , BC ≥ 0.0017 , and CC ≥ 0.2992 , which were the key targets of quercetin for RA treatment. These key target proteins were sorted following the degree value, and the topmost 10 targets are taken as the core targets, namely SRC, HSP90AA1, MAPK1, PIK3R1, HRAS, AKT1, MAPK8, RHOA, MAPK14, and ESR1 (Figure 3).

3.4. GO and KEGG Enrichment Analyses

Quercetin anti-RA targets were enriched in 634 GO items (all $p < 0.05$), containing 456 biological processes (BP), 61 cellular components (CC), as well as 117 molecular function (MF). The topmost ten items of BP, CC, along with MF were chosen to plot a histogram (Figure 4).

According to the results of the GO analysis, we found that:

In biological processes (BP), the protein phosphorylation, proteolysis, and negative regulation of apoptotic process have the most targets ;

Among the cellular components (CC), the extracellular region, cytosol, and extracellular exosomes have the most targets;

In terms of molecular function (MF), the identical protein binding, protein tyrosine kinase activity, and ATP binding have the most targets.

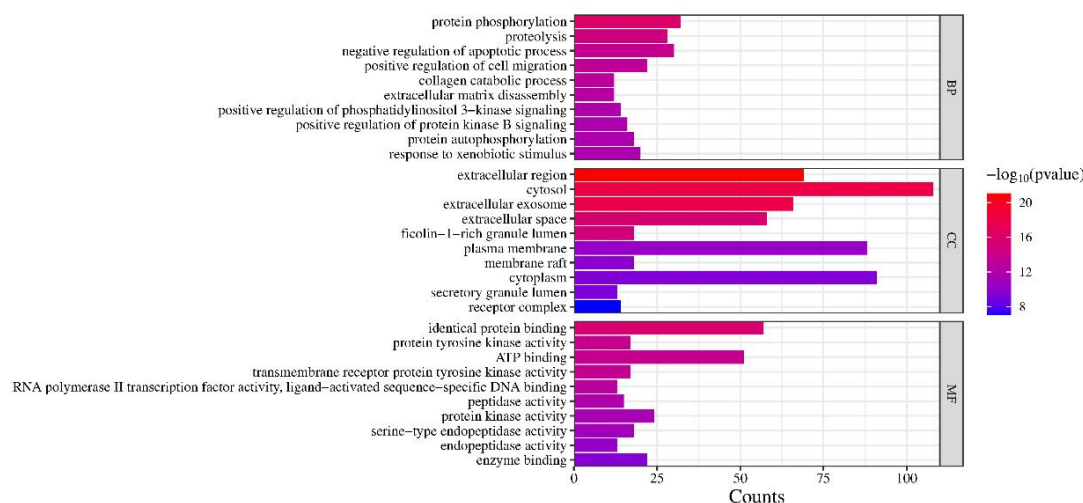


Figure 4. Go function analysis.

In the meantime, KEGG enrichment analysis yielded 142 signaling pathways; Figure 5 presents a bubble diagram of the topmost 20 pathways.

The PI3K-Akt pathway is displayed in Figure 6.

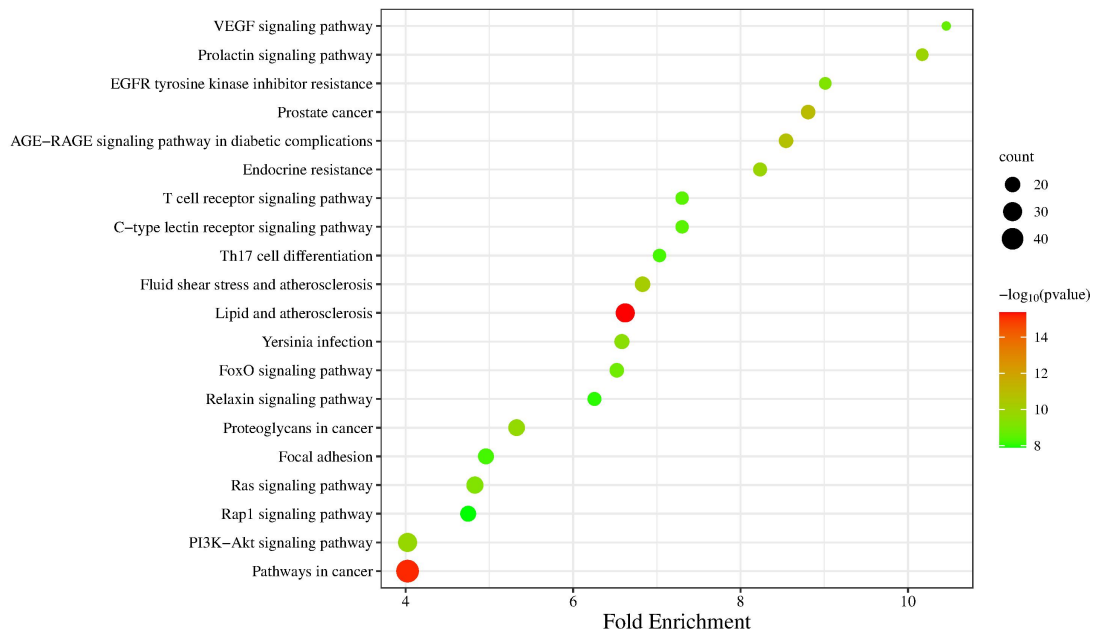


Figure 5. KEGG pathway analysis.

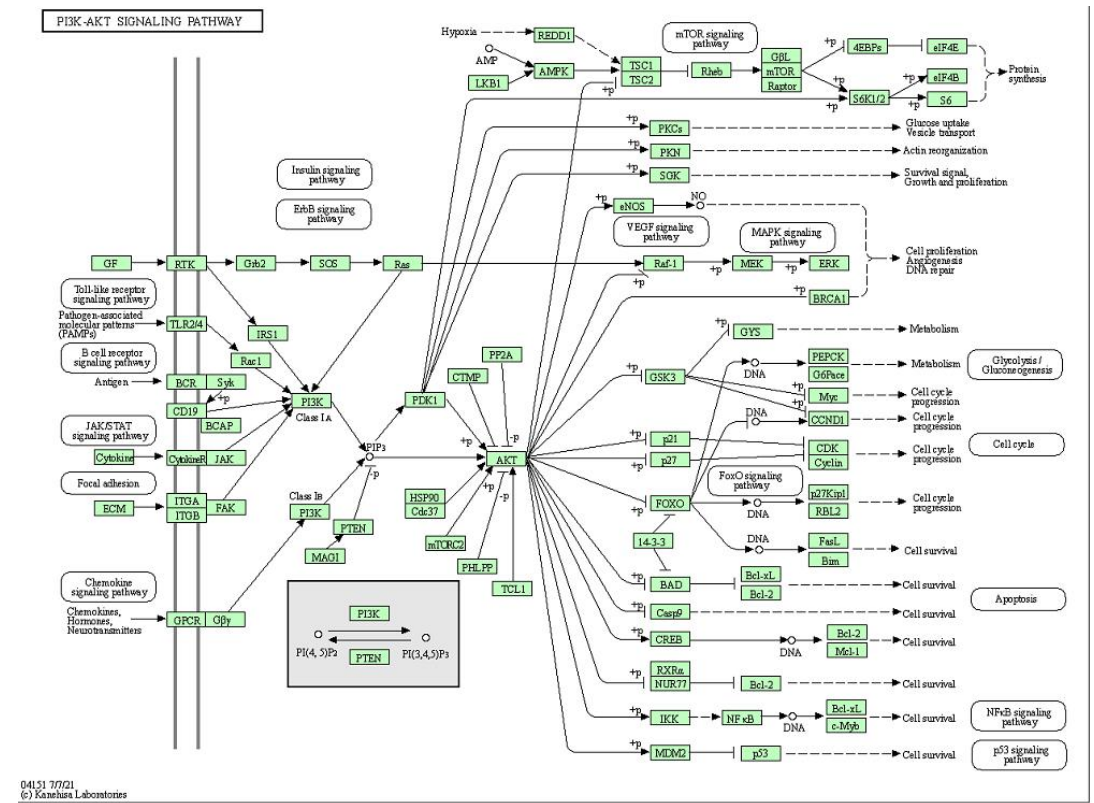


Figure 6. PI3K-Akt signaling pathway.

3.5. Drug-Target-Pathway-Disease Network

The implementation of the Cytoscape 3.9.1 software was for establishing the drug-target-pathway-disease network (Figure 7). In the figure, yellow diamonds represented quercetin, purple circles indicate RA, blue squares refer to targets, green triangles represent signaling pathways, and gray lines correspond to interactions between them.

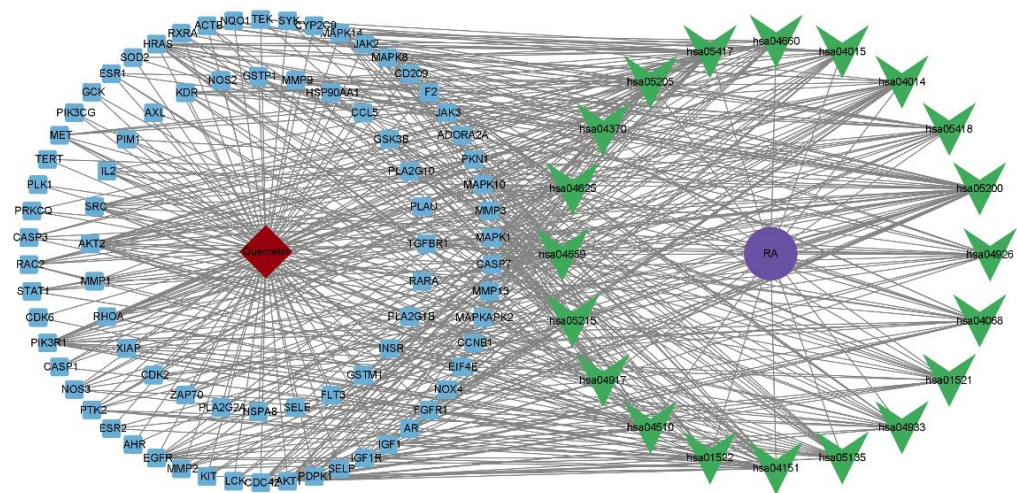


Figure 7. Drug-target-pathway-disease network.

3.6. Molecular Docking

The pivotal targets are sequentially docked with quercetin (Figure 8).

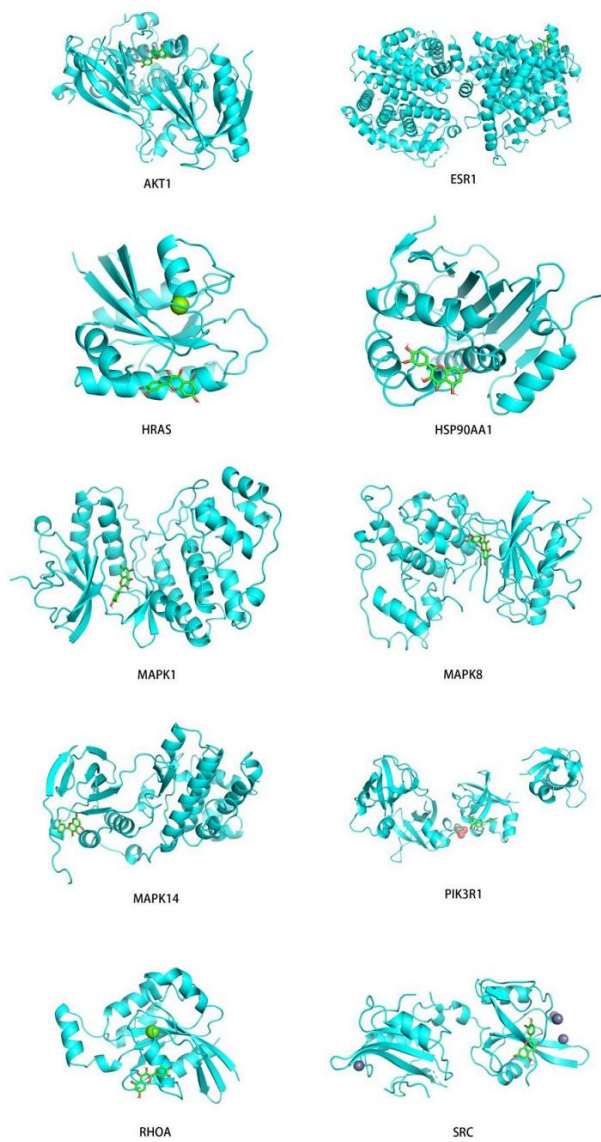


Figure 8. Molecular docking.

The assessment of the binding activity could be achieved based on the binding energy. A value below 0 signified that they could be combined spontaneously. Reduced binding energy reflected easy binding. Generally, binding energy below $-5.00 \text{ kcal} \cdot \text{mol}^{-1}$ indicates favorable binding activity. In our work, Table 1 exhibits the molecular docking's binding energy, with $-8.0 \text{ kcal} \cdot \text{mol}^{-1}$ average.

Table 1. Molecular docking's binding energy ($\text{kcal} \cdot \text{mol}^{-1}$).

SRC	HSP90AA1	MAPK1	PIK3R1	HRAS	AKT1	MAPK8	RHOA	MAPK14	ESR1
-6.9	-9.1	-7.3	-7.3	-6.2	-10.4	-9.7	-7.6	-8.9	-7.0

4. Discussions

RA can usually cause progressive joint damage, loss of function, and complications. Studies have found that RA existed in Native Americans thousands of years ago, but it did not appear in Europe until the 17th century [9]. The prevalence of RA is related to heredity, gender, and race [2], and is generally higher in industrialized countries [10]. Drugs for the treatment of RA are basically assigned into 4 categories: glucocorticoids (GCs), disease-modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), as well as biological drugs [11], among which, symptomatic treatment with NSAIDs and DMARDs are two principal approaches for RA therapy. Untreated RA leads to joint destruction through cartilage and bone erosion, eventually leading to loss of body function, and therefore, early treatment is essential for controlling disease activity and avoiding joint destruction, which can be avoided or greatly slowed down in 90% of patients with progression of joint damage [12].

Recently, quercetin has been gradually applied in RA therapy [13], and its possible mechanisms include immune regulation [14], anti-inflammatory [15], and bone protection. Nevertheless, the signaling pathways, potential targets, along with molecular mechanisms of quercetin against RA remain to be further studied. Network pharmacology expounds drug-target interactions in terms of multi-purpose level and multi-perspective interaction networks, and also discloses the drug's action within the theoretical framework of diseases, drugs, as well as targets. Based on this, this research is intended to discuss the quercetin's promising targets and molecular mechanisms in the prevention of RA from a microscopic viewpoint via network pharmacology and molecular docking, thus providing new directions for the pathogenesis and drug therapy of RA.

4.1. Target Analysis

Searching the database yielded 372 drug targets and 3,418 disease targets, consisting of 187 crossover targets and 10 core targets, namely SRC, HSP90AA1, MAPK1, PIK3R1, HRAS, AKT1, MAPK8, RHOA, MAPK14, and ESR1.

The findings of molecular docking revealed that all binding energy below $-5 \text{ kcal} \cdot \text{mol}^{-1}$, implying that quercetin could steadily bind to receptor proteins and reflect therapeutic effects. Quercetin had the lowest binding energy to AKT1, which showed the best docking effect. Meanwhile, the binding energies of MAPK8, HSP90AA1, and MAPK14 were also low, which were vital core targets of quercetin for anti-RA.

AKT1

Protein kinase B (PKB, namely AKT) is a serine/threonine protein kinase. There are three subtypes in the Akt family: Akt1 (α), Akt2 (β), and Akt3 (γ), among which,

Akt1 is the most commonly expressed subtype in mammalian cells and tissues. Akt is the downstream target of phosphatidylinositol 3-kinase (PI3K) [16]. The activated Akt functions in metabolism, transcription, cell migration, cell cycle arrest, cell survival, and angiogenesis.

MAPK

MAPK is a group of serine/threonine protein kinases that contains p38 mitogen-activated protein kinase (p38MAPK α , β , γ , and δ), extracellular signal-regulated kinase 1/2 (ERK1/2), as well as c-Jun N-terminal kinase 1/2/3 (JNK1/2/3) [17]. The activation pathway of MAPK is a typical three-level enzymatic cascade reaction. Upon the binding of the upstream growth factor to a specific receptor, MAPK regulates its downstream transcription factors through the gradual phosphorylation of MAPKKK-MAPKK-MAPK, thereby mediating many physiological activities, such as inflammation, proliferation, differentiation, apoptosis, cancerization, invasion and metastasis of tumor cells. Besides, the MAPK pathway activation mediates the expression of pro-inflammatory cytokines, which is a key pathway for the development of RA. Numerous experimental articles have disclosed that quercetin can restrain the expression of the MAPK pathway [18].

MAPK8, also called c-JUN N-terminal kinase (JNK), is of great significance in the induction of the gene expression of cytokine and matrix metalloproteinase (MMP) in synoviocytes. JNK1 and JNK2 are the two subtypes of the JNK family. JNK1 has been found to promote osteoclast differentiation. In the absence of JNK1, osteoclast progenitors are not yet mature to perform bone resorption and differentiate into osteoclasts [19]. JNK inhibitors can effectively control RA by reducing ROS production, reducing IL-6 levels, and down-regulating MMP-3 expression, while quercetin can exert an anti-apoptotic effect by interfering with the JNK pathway.

MAPK14 is the α subtype of p38, and the α and γ subtypes of p38 MAPK are dominant in chronic inflammation. p38 is highly expressed and activated in the synovial tissues of RA. SB203580, a commonly used p38 MAPK inhibitor, is able to decrease the secretion of pro-inflammatory mediators in monocytes/macrophages, neutrophils, together with T lymphocytes. MAPK14 plays a direct and important part in alleviating the inhibitory control of autophagy on inflammation [20].

HSP90AA1

HSP90AA1 belongs to the HSP90 family and an essential heat shock protein. Heat shock proteins (HSPs), consisting of HSP70 and HSP90 families, are omnipresent and phylogenetically conserved stress response molecules that mainly participate in maintaining protein balance. HSP90AA1 can promote autophagy through the PI3K/Akt/mTOR pathway and also impede apoptosis via the JNK/P38 pathway. Some scholars have found that HSP90 may be a potential biomarker related to autophagy defects in OA [21].

4.2. Pathway Analysis

PI3K-Akt

The PI3K-Akt pathway is a key pathway involved in the inflammatory response and bone metabolism imbalance. The activated PI3K-AKT signal will be transmitted to the downstream pathways, thereby resulting in the activation of many pathways, including cell cycle, apoptosis, protein translation, and P53 access.

The PI3K-Akt pathway stimulates the expression of inflammatory mediators, participates in the abnormal proliferation of fibroblast-like synoviocytes (FLS), inflammation of the synovium, differentiation, and generation of osteoclasts, and chemotactic migration of osteoclasts to destroy bone and articular cartilage, eventually leading to joint deformity and ankylosis. Treatment of a mouse model of collagen-stimulated arthritis with LY294002, the PI3K-Akt pathway's specific inhibitor, is capable of improving symptoms such as synovial cell proliferation and inflammatory cell infiltration in the ankle joint [22].

Abnormal activation of the PI3K-Akt pathway also boosts HIF-1 α and VEGF expression, enhances RA angiogenesis, prevents the bone from acquiring nutrients via the synovium, and takes part in the secretion of diverse inflammatory mediators and proteolytic enzymes, with the corrosion of subchondral bone, ligaments, tendons, and other tissues, resulting in damage of articular cartilage, subchondral osteolysis, and worsening the RA course. In vivo and in vitro assays unveiled that blockage of the PI3K-Akt pathway can improve RA angiogenesis [23].

In addition, many experiments have substantiated that the PI3K-Akt pathway is linked to programmed cell death (PCD) [24], and blocking the PI3K-Akt pathway can inhibit cell apoptosis and ferroptosis, and promotes autophagy [25] to relieve RA symptoms.

RAS Signaling Pathway

Under normal circumstances, the combination of Ras and GDP is in an inactive state, but when stimulated by an external guanine nucleotide exchange factor (GEF), Ras and guanosine triphosphate (GTP) are activated. GTP-Ras phosphorylates Raf, causing the activation of downstream MEK/ERK. Ras/Raf/Mek/Erk is a classic pathway of the MAPKs subfamily, which affects abnormal cell proliferation, hypertrophy, and differentiation.

The Ras/Raf/MEK/ERK signaling pathway activation can activate ERK and directly enhance the up-regulation of autophagy marker proteins LC3 and p62 for the initiation of autophagy; besides, it can lead to the down-regulation of lysosomal-associated membrane protein 1 (LAMP1) and LAMP2, which prevents the combination of autophagosomes and lysosomes, thereby inhibiting the autophagosome degradation. Chen et al.[26] proved through experiments that blocking the Ras/MEK/ERK pathway is one of the key mechanisms for Baihu Jia Guizhi Decoction to improve RA synovitis. Su et al. [27] demonstrated that YuXueBi tablet can inhibit angiogenesis by inhibiting the LOX/Ras/Raf-1 pathway, thereby reducing RA disease severity and reduce bone erosion.

FOXO Signaling Pathway

FoxO is mainly regulated by two signaling pathways: PI3K/AKT signaling pathway; JNK signaling pathway. It plays a role in DNA damage repair, oxidative stress, cell apoptosis, autophagy, proliferation, differentiation, and metabolism. Studies have shown that suppression of PI3K/AKT and MEK/ERK pathways enhances FOXO transcriptional activity in synergistic action, thereby inhibiting cell migration and capillary formation [28].

VEGF Signaling Pathway

VEGF is a very important pro-angiogenic factor that is able to enhance endothelial cell viability and migration, and it can also promote the secretion of interstitial collagenase, degrade the cell matrix, and exert function in angiogenesis.

The pathogeny and pathological changes of RA are closely related to VEGF [29]. VEGF is up-regulated in RA patients' serum and synovia fluid, and the VEGF value is positively correlated with the disease activity of RA. Cytokines and growth factors, such as IL-34 [30], placental growth factor (PIGF), TNF- α , and IL-6, indirectly execute the function of neovascularization or anti-angiogenesis by regulating the expression of VEGF. VEGF is a prospective therapeutic target in RA patients [31], and suppression of the VEGF signaling pathway can inhibit angiogenesis in rheumatoid synovium.

RAP1

Rap1 belongs to the small-molecular-weight GTPase of the Ras family, and Rap1 exerts functions in stimulating angiogenesis and maintaining blood vessel stability. Through animal experiments, some scholars believe that restoring the function of Rap1 in RA synovial T cells may be helpful for RA therapy [32].

5. Conclusions

In this study, 372 drug targets and 3,418 disease targets were acquired by means of network pharmacology, including 187 cross-targets and 10 core targets, which were validated by molecular docking. Quercetin may modulate PI3K-Akt, Rap1, FoxO, and Ras signaling pathways in either a direct or an indirect way by regulating the expression of HSP90AA1, AKT1, MAPK8, MAPK14, and other protein targets, thereby reducing chondrocyte apoptosis, alleviating immune inflammatory response, improving synovial hyperplasia, and inhibiting angiogenesis.

This study has laid a favorable theoretical foundation for further exploration of RA therapy with quercetin. Although our study reached the goal, the adopted method still has certain limitations and warrants further clinical and experimental validation.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Data Availability

The figures and tables used to support the findings of this study are included with in the article, and the original data are available from the first author or corresponding author upon request.

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