Traditional Chinese Medicine Drynariae Rhizoma and Cuscuta Chinensis Suppress Osteoarthritis by Quercetin-AKT1 and Luteolin-IL6/VEGFA Direct Binding

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Research

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Abstract

Background: Drynaria Fortunei and Cuscuta Chinensis are among the most used traditional Chinese medicine herbal prescriptions and have a significant therapeutic effect on osteoarthritis. However, the purpose of this study intends to elaborate the molecular mechanism of action through network pharmacology. The active ingredients of TCM and the potential targets for the treatment of osteoarthritis were selected through the TCMSP, OMIM and Genecards.

Results: The 27 components and 85/117 targets of Drynaria Fortunei and/or Cuscuta Chinensis were identified for osteoarthritis. Pharmacological and PPI network analysis identified top 3 active components (kaempferol, luteolin, and quercetin) and core proteins (IL6, AKT1, and VEGFA). GO and KEGG analysis identified the top 3 functions (cytokine and cell/nuclear receptor) and pathways (PI3K-Akt, TNF and IL-17). Molecular docking showed strong binding ability between quercetin-AKT1 and luteolin-IL6/VEGFA. Interaction analysis mapped the quercetin-AKT1 and luteolin-IL6/VEGFA binding to specific hydrogen and hydrophobic bonds.

Conclusions: The main active components, common target proteins, functional activities, and signaling pathways of TCM Drynaria Fortunei and Cuscuta Chinensis for the treatment of osteoarthritis were identified by Network pharmacology. We found, for the first time, that drynariae rhizoma and cuscuta chinensis suppress osteoarthritis by quercetin-AKT1/IL6 and luteolin-VEGFA direct binding. Our findings have significant implication for our understanding of the molecular mechanism of action in the treatment of osteoarthritis and future development of osteoarthritis treatment using quercetin and luteolin.

Introduction

Traditional Chinese Medicine (TCM) has been developed for thousands of years and has shown the advantages of safety, low cost, and obvious effect. The treatment of osteoarthritis by TCM has grown to be a hot spot [1]. Due to the complexity of TCM ingredients, each TCM contains numerous compounds that take effects on different targets in multiple signaling pathways. Drynaria Fortunei and Cuscuta Chinensis have a long history as traditional Chinese herbal medicines, which have been shown to have a significant effect on osteoarthritis [2; 3]. Drynaria Fortunei is commonly used to treat bone fractures and bone metabolic disorders, such as osteoarthritis [4]. Studies have shown that Drynaria Fortunei is effective for bone repair after trauma. Yang RC et al found that Gusuibu (Drynaria Fortunei) inhibits the NF-κB/IκBα/IKK signaling pathway, which induces down-regulation of the expression of cell adhesion molecules and chemokines [5]. Researches have also shown that the flavones in Drynaria Fortunei can resist osteoarthritis by a variety of mechanisms [6]. Cuscuta, which has produced anti-inflammatory effects, is commonly used to treat chronic waist pain and knee soreness [7]. We know that osteoporosis is an unfavorable factor in inducing the formation of osteoarthritis. Wegiel B et al. have shown that dodder seed (Cuscuta Chinensis) increased the production of two key extracellular matrix proteins, collagen I and laminin B2 [8], which means that Dodder seed alone may have the potential to prevent osteoporosis. Another study showed that Cuscuta Chinensis alcohol extracts are able to promote the growth of

osteoblasts and prevent osteoarthritis [9]. Due to many chemical components of traditional Chinese medicine, further research needs to be conducted to screen anti-osteoarthritis active ingredients and their targets.

Osteoarthritis is a common complex chronic degenerative disease that is closely related to age and weight [10]. It is characterized by the destruction of articular cartilage, lesions of subcortical cartilage, and other perpendicular tissues, increasing the rate of disability and decreasing the quality of life due to pain [11]. Osteoarthritis affects 240 million people globally, with about 10% of men and 18% of women over 60 years old diagnosed with osteoarthritis [12]. The prevalence of symptomatic osteoarthritis in China is about 15% currently and is expected to jump to 35% by 2030 [13]. With the improvement of medical care and national living standard, the aging of the population is becoming increasingly pronounced, and with the obesity index keeps rising, osteoarthritis has become the major heart disease of human society. Osteoarthritis and other elderly related diseases (such as heart disease, chronic obstructive pulmonary disease, post-menopausal osteoporosis, and fractures) cast an ominous shadow over patients and the whole society. The main applications of clinical intervention for osteoarthritis nowadays include weight loss, anti-inflammation, oral analgesics, physical therapy, intra-articular injection of hypertonic acid, joint replacement and Chinese herbal medicines, which showed significant anti-osteoarthritis effects [14].

Network pharmacology is able to overcome the defect of the traditional research model of a definite target gene and signaling pathway [15], and provides the possibility of comprehensively expounding the molecular mechanism of the compounds in Chinese medicine in treating diseases. In the present study, based on the network pharmacology analysis method of potential targets of TCM compounds, the molecular mechanisms of the treatment of osteoarthritis with Chinese herbal prescription of Drynaria Fortunei and Cuscuta Chinensis are expounded comprehensively, which will provide guidance for clinical applications.

Materials And Methods

Screening of Effective Components and targets of Drynaria Fortunei and Cuscuta Chinensis

First of all, the components of Drynaria Fortunei and Cuscuta Chinensis were selected from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) (http://lsp.nwu.edu.cn/tcmsp.php) [16]. Then, the effective components and their target proteins of Drynaria Fortunei and Cuscuta Chinensis were obtained by the screening set as oral bioavailability (OB) ≥ 30% and drug-like (DL) ≥ 0.18 [17]. Last, with the help of Perl software (V5.30.1.1), the target proteins of Drynaria Fortunei and Cuscuta Chinensis were further screened for the human proteins in UniProt database (https://www.uniprot.org/).

Identification of osteoarthritis related targets

Genecards (https://genealaart.genecards.org/) and OMIM (http://omim.org/) databases were combined and searched for all the proteins related to osteoarthritis. The target proteins of the related traditional Chinese medicine may not be found in osteoarthritis related proteins in the two databases of Genecards and OMIM since the Chinese medicine target proteins were obtained by searching the TCMSP database with the effective compounds.

Identification of TCM-osteoarthritis common target proteins

The targets related to osteoarthritis and the targets of the effective compounds of Drynaria Fortunei and Cuscuta Chinensis were intersected by using R software (V3.5.1) to identify, and the wainscot (Venn diagram) of Drynaria Fortunei–osteoarthritis targets and Cuscuta Chinensis–osteoarthritis common targets were created.

Construction of PPI and pharmacological network of the common targets

Input Drynaria Fortunei/Cuscuta Chinensis and Osteoarthritis common targets in String's online website (https://string-db.org/), obtain the network diagram of the interaction among the targets, establish the confidence level of 0.4, and export the TSV file for later use. Network analyzer, a tool in Cytoscape software V3.7.1 (http://www.cytoscape.org/), was used to obtain the protein-protein interaction (PPI) and pharmacological network of Drynaria Fortunei and Cuscuta Chinensis for the treatment of osteoarthritis. We can directly observe which components and proteins in Drynaria Fortunei and Cuscuta Chinensis are critical in the treatment of osteoarthritis.

Identification of core targets in the PPI Network

The common target genes of Drynaria Fortunei/Cuscuta Chinensis and osteoarthritis were mapped by Perl software. R software was utilized to calculate the number of interactions between major proteins for identification of core targets, and the bar graph of the core targets from the protein interaction network was plotted. Network analyzer, a tool in Cytoscape software V3.7.1 (http://www.cytoscape.org/), was used for further PPI network analysis, and the size and color of nodes were placed to reflect the size of Degree value, or the number of the interactions, and a visualized PPI network diagram is presented.

Functional enrichment analysis

GO functional enrichment analysis and KEGG pathway enrichment analysis of the common target proteins were performed to identify their biological functions and signaling pathways in the treatment osteoarthritis with Drynaria Fortunei and Cuscuta Chinensis. P-value < 0.05 was set as statistically significant.

Molecular docking

AutoDock Tools 1.5.6 software was used to delete water molecules in AKT1, IL6, and VEGFA, separate ligands and receptors, add non-polar hydrogen, calculate a Gasteiger charge, and save the results as a pdbqt file. The small molecule 3D coordinate file is downloaded from the PubChem website. After

checking the spatial structure in the Pymol software, and then converted to pdb format using the Openbabel plug-in function. Load structural parts into the AutoDock Tools 1.5.6 program, add atomic charges, assign atomic types, and all flexible keys can be rotated by default. Save as pdbqt format as docking ligand. AKT1, IL6, and VEGFA were used as receptors, and the candidate components of tranditional Chinese medicine were used as ligands, and the active site of molecular docking was determined according to the coordinates of the ligand in the target protein complex. Gridbox coordinates and size were set according to AKT1, IL6, and VEGFA active pockets. AutoDock Vina was used for molecular docking, and the docking results were analyzed and processed. The lowest binding energy was used as the highest binding affinity of the docking of the target protein and ligand. Observation and mapping were performed using Pymol and Ligplot 1.4.5 softwares.

Results

The 27 components and 85/117 targets of Drynaria Fortunei and/or Cuscuta Chinensis were identified for osteoarthritis

With the OB ≥ 40% and DL ≥ 0.19, a total of 27 eligible active components of Drynaria Fortunei and Cuscuta Chinensis (Table 1) and 202 targets corresponding to the active components were obtained from the TCMSP database.

Table 1
Effective components of Drynaria Fortunei and Cuscuta Chinensis.

MOL ID	Chemical name	OB(%)	DL
MOL005190	Eriodictyol	71.79	0.24
MOL009087	Marioside_qt	70.79	0.19
MOL005944	Matrine	63.77	0.25
MOL009078	Davallioside A_qt	62.65	0.51
MOL000569	Digallate	61.85	0.26
MOL004328	Naringenin	59.29	0.21
MOL001558	Sesamin	56.55	0.83
MOL006649	Sophranol	55.42	0.28
MOL000492	(+)-catechin	54.83	0.24
MOL001978	Aureusidin	53.42	0.24
MOL000354	Isorhamnetin	49.6	0.31
MOL000098	Quercetin	46.43	0.28
MOL000449	Stigmasterol	43.83	0.76
MOL005440	Isofucosterol	43.78	0.76
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) Chroman-4-one	42.36	0.21
MOL000422	Kaempferol	41.88	0.24
MOL009063	Cyclolaudenol acetate	41.66	0.79
MOL002914	Eriodyctiol (flavanone)	41.35	0.24
MOL009091	Xanthogalenol	41.08	0.32
MOL009075	Cycloartenone	40.57	0.79
MOL000184	NSC63551	39.25	0.76
MOL009061	22-Stigmasten-3-one	39.25	0.76
MOL009076	Cyclolaudenol	39.05	0.79

Notes: MOL, molecule; OB, oral bioavailability; DL, drug-like.

MOL ID	Chemical name	OB(%)	DL
MOL000953	CLR	37.87	0.68
MOL005043	campest-5-en-3beta-ol	37.58	0.71
MOL000358	Beta-sitosterol	36.91	0.75
MOL000006	Luteolin	36.16	0.25
Notes: MOL, molecule; OB, oral bioavailability; DL, drug-like.			

By using R language software, the osteoarthritis related targets (2873) were intersected with the Drynaria Fortunei (139) and Cuscuta Chinensis (187) targets, respectively, and the wiring diagram of the common target of TCM-disease was built (Fig. 1). The 85 and 117 common target proteins of osteoarthritis and Drynaria Fortunei/Cuscuta Chinensis were identified, respectively.

Pharmacological and PPI network analysis identified the top 3 active components (kaempferol, luteolin, and quercetin) and core proteins (IL6, AKT1, and VEGFA)

The pharmacological and PPI networks of the 27 active components and the 134 common target proteins of osteoarthritis and Drynaria Fortunei/Cuscuta Chinensis were shown in Fig. 2. Based on the connectivity of proteins in the PPI network, the top 3 active components were: kaempferol, luteolin, and quercetin (Fig. 2).

As shown in Fig. 3a, the PPI network contains 134 nodes and 2640 edges, with an average node degree of 39.4 and an average local clustering coefficient of 0.665. The number of adjacent nodes represent the probability of growing to be a core protein. According to the number of adjacent nodes, the 30 core proteins TCM-osteoarthritis were IL6，AKT1，VEGFA，MAPK3，JUN，PTGS2，CASP3，MAPK8，EGF，EGFR，MAPK1，MMP9，CXCL8，MYC，IL1B，CCL2，CCND1，CAT，ESR1，IL10，FOS，MAPK14，MMP2，ICAM1，PPARG，ERBB2，HMOX1，IL4，RELA，SERPINE1 (Fig. 3a-b). The results also demonstrate that the top 3 proteins for the treatment of osteoarthritis by Drynaria Fortunei and Cuscuta Chinensis were IL6, AKT1, and VEGFA (Fig. 3a-b).

GO and KEGG analysis identified the top 3 functions (cytokine and cell/nuclear receptor) and pathways (PI3K-Akt, TNF and IL-17)

The results of GO enrichment analysis of the core targets of Drynaria Fortunei and Cuscuta Chinensis in the treatment of osteoarthritis are shown in Fig. 4. The results showed that the targeted proteins are mainly related to 20 biological processes, which mainly involves cytokine activity, cell receptor binding, nuclear receptor activity, receptor ligand activity, transcriptional activation activity, RNA polymerase II area sequence-specific DNA-binding, proximal promoter sequence-specific DNA binding and protein heterodimerization.

KEGG pathway enrichment analysis results of the core targets for the treatment of osteoarthritis by Drynaria Fortunei and Cuscuta Chinensis are shown in Fig. 5. The KEGG pathways mainly involves PI3K-Akt signaling, TNF, IL-17 signaling, MAPK signal, cell apoptosis, endocrine resistance, c-type lectin receptors signaling, toll-like receptors signaling, Th17 cell differentiation, cell aging, T cell receptors signaling, HIF-1 signaling, osteoclast differentiation, FoxO signaling, platinum resistance, differentiation of Th1 and Th2 cells, EGFR tyrosine kinase inhibitor-resistance and the NF-κB signaling.

Molecular docking showed strong binding ability between quercetin-AKT1 and luteolin-IL6/VEGFA

The top 3 core proteins AKT1, IL6, and VEGFA showed the most connection in the interaction network (Fig. 3a). To further validate our findings above, the top 3 core proteins were molecularly docked with the top 3 active components (kaempferol, luteolin, and quercetin) through AutoDock Vina software. We know that a low absolute value of the minimum binding energy means high binding activity between the target protein and compounds and results in better function of the active compounds. The binding energy of less than-5 kcal/mol indicates that active compound and target protein have strong binding ability. We found that kaempferol was not related to the top 3 core target proteins (Table 2). Interestingly, we found that among them, AKT1-quercetin (Fig. 6a), IL6-luteolin (Fig. 6b), VEGFA-luteolin (Fig. 6c) have the lowest binding energy of -9.5, -7.2, -5.8 kcal/mol, respectively. Taken together, these results imply that the quercetin and luteolin in Drynaria Fortunei and Cuscuta Chinensis have a strong binding ability respectively with AKT1 and IL6/VEGFA, suggesting that quercetin and luteolin in Drynaria Fortunei and Cuscuta Chinensis are the two major compounds in the treatment of osteoarthritis by targeting AKT1 and IL6/VEGFA, respectively.

Table 2
Drug and target docking parameters and corresponding calculation results.

Target name	PDB ID	Compound	(X×Y×Z)/nm^3	Center (X,Y,Z)	Affinity of compound (kcal/mol)
AKT1	4EJN	Kaempferol	22.50x22.50x22.50	35.39x43.72x18.44	-9.1
		Luteolin	22.50x22.50x22.50	35.39x43.72x18.44	-9.4
		Quercetin	22.50x22.50x22.50	35.39x43.72x18.44	-9.5
IL-6	1ALU	Kaempferol	22.50x22.50x22.50	(-7.51)x(-12.83)x0.06	-6.5
		Luteolin	22.50x22.50x22.50	(-7.51)x(-12.83)x0.06	-7.2
		Quercetin	22.50x22.50x22.50	(-7.51)x(-12.83)x0.06	-7.0
VEGFA	4KZN	Kaempferol	41.25x41.25x41.25	(-32.07)x(-103.23)x(-4.38)	-5.4
		Luteolin	41.25x41.25x41.25	(-32.07)x(-103.23)x(-4.38)	-5.8
		Quercetin	41.25x41.25x41.25	(-32.07)x(-103.23)x(-4.38)	-5.7

Interaction analysis mapped the quercetin-AKT1 and luteolin-IL6/VEGFA binding to specific hydrogen and hydrophobic bonds

Interaction analysis was based on Ligplot 1.4.5, the formation of hydrogen bonds, hydrophobic interaction, and structural comparison was automatically calculated (Fig. 6d-f). The interaction sites of AKT1 and quercetin are shown in Fig. 6d, where the ligand hydroxyl group forms hydrogen bonds with residues Ser205, Tyr272, Asp292, and Thr82. Other hydrophobic residues such as Lys268, Trp80, Val270, and Val271 form a strong stacking effect with the hydrophobic ligand ring that is conducive to the binding. The interaction residues of IL6 and luteolin are shown in Fig. 6e, where the hydroxyl group of the ligand forms a hydrogen bond with Arg179, Gln175, and Asp34. Hydrophobic residues Leu178, Arg30, and Leu30 form a strong stacking effect with the hydrophobic ligand ring to stabilize the binding. The interaction residues of VEGFA and luteolin are shown in Fig. 6f, in which the hydroxyl group of the ligand forms a hydrogen bond with Ser24 and Val15. Other hydrophobic residues such as Asn62, Cys60, Tyr21, Phe17, Val20, Lys16, etc., form a strong stacking effect with the hydrophobic ligand ring to stabilize the binding.

Discussion

It has been shown that the dry rhizome of Drynaria Fortunei has the effects of strengthening bone, relieving pain, regulating lipid metabolism, and curbing inflammation [18]. Cuscuta Chinensis is a family

of plants in the family spinose, which has the effects of nourishing the liver and kidney and relieving fetal diarrhea [19]. Relevant studies have shown that Drynaria Fortunei and Cuscuta Chinensis play an important role in inhibiting the expression of positive factors [20], and have shown an effect against osteoarthritis. However, previous studies mainly focused on the functional analysis of osteoblasts and osteoclasts, and few of them have uncovered the molecular mechanism. Therefore, it is of great importance to study the molecular mechanism of Drynaria Fortunei and Cuscuta Chinensis against osteoarthritis.

In this study, we showed that the principal anti-osteoarthritis chemical components in Drynaria Fortunei and Cuscuta Chinensis are kaempferol, luteolin, and quercetin. Previous studies have demonstrated that kaempferol induces autophagy in osteoblasts by inhibiting adipogenesis, inflammation, oxidative stress, autophagy in osteoclast, and apoptosis in osteoblasts [21]. Bone protection is obtained as a result of regulating estrogen receptors, bone morphogenetic protein 2 (bmp-2), nuclear factor-b (NF-κB), mitogen-activated protein kinase (MAPK), and mammalian target mediated rapamycin (mTOR) signaling pathways [21]. Kaempferol inhibits IL-1β-stimulated, RANKL-mediated osteoclast differentiation, it indicates that kaempferol has an inhibitory role in the bone loss by preventing osteoclast formation [22]. Luteolin effectively inhibits the proliferation of osteoarthritis chondrocytes by down-regulation the expression of JNK, p38, and MAPK in osteoarthritis chondrocytes, and also reduce the inflammatory response, protect chondrocytes, and delay cartilage degeneration by down-regulation of NO, TNF- and IL-6 [23]. Quercetin inhibits osteoclast activation and reduces bone destruction by suppressing RANKL/RANK/OPG signaling pathway [24]. In an *in vivo* experiment, in which osteoarthritis was induced by surgery in rabbits, Wei B et al. [25]revealed that quercetin improves the degeneration of osteoarthritis by weakening the oxidative stress response and inhibiting the degradation of Chondro extracellular matrix through up-regulate SOD and timp-1 and down-regulate mmp-13. We found, for the first time, that drynariae rhizoma and cuscuta chinensis suppress osteoarthritis by their components of quercetin and luteolin.

The results of the present study showed that Drynaria Fortunei and Cuscuta Chinensis mainly target IL6, AKT1, and VEGFA against osteoarthritis. IL-6 has been revealed to associate with hip osteoarthritis pain and has been found in the synovial fluid of patients with hip osteoarthritis [26]. Some experimental studies have found that curcumin can reduce the IL-6 content of osteoarthritis rat, by blocking the TLR4/NF-κB signaling pathway to reduce the level of inflammation in osteoarthritis and prevent their knee injuries [27]. Studies have shown that when the expression of GRK5 is hindered in patients with osteoarthritis, the expression level of IL-6 and NF-κB also were down-regulated. Another research also demonstrates that IL-6 regulates the degradation of chondrocytes through the NF-κB signaling pathway [28]. In the experimental study of curcumin, curcumin treatment can reduce the expression of IL-6 and TNF-α in rat osteoarthritis, thereby blocking the NF-κB signaling pathway and protecting knee osteoarthritis [27]. Research showed that Mir-495 is highly expressed in the cartilage of patients with osteoarthritis, which repressed the expression of AKT1 and results in the inhibition of cell proliferation and apoptosis of

chondrocytes [29]. In mice with surgically induced osteoarthritis, calcified osteophyte formation, but not cartilage degradation, was prevented in the Akt1$^{(-/-)}$ joints. AKT1 in chondrocytes promotes the calcification of cartilage in osteoarthritis [30]. The vascular endothelial growth factor (VEGF) family has been shown to play a key role in controlling articular cartilage catabolism and angiogenesis, which is a vital step in mesenchyme progenitor cell endochondral ossification [31]. Extensive studies have proved that inhibition of VEGFA signaling reduces child progression of osteoarthritis. VEGFA is currently recognized as an angiogenic agent that promotes endothelial cell proliferation and migration. Studies have shown that VEFGA is associated with cartilage destruction in osteoarthritis, which directly up-regulates the expression of VEGF through the PI3K/AKT pathway, thus aggravating the destruction of bone joints[32]. Other studies showed that ricolinostat protects the articular cartilage of osteoarthritis patients by down-regulating the expression of VEGFA and then the PI3K/AKT signaling pathway thus was inhibited[33]. In combination with our current study, we showed, for the first time, that Drynaria Fortunei and Cuscuta Chinensis suppressed the development of osteoarthritis by inhibiting IL-6, AKT1, and VEGFA.

In addition, our molecular docking showed strong binding ability between quercetin-AKT1 and luteolin-IL6/VEGFA. Interaction analysis mapped the quercetin-AKT1 and luteolin-IL6/VEGFA binding to specific hydrogen and hydrophobic bonds. Therefore, we showed using multiple methods, for the first time, that Drynaria Fortunei and Cuscuta Chinensis suppressed the development of osteoarthritis by quercetin-AKT1 and luteolin- IL6/VEGFA direct binding.

Conclusion

In this study, we used data mining and network pharmacology to explore the possible molecular mechanism of Drynaria Fortunei and Cuscuta Chinensis in the treatment of osteoarthritis. We found, for the first time, that drynariae rhizoma and cuscuta chinensis suppress osteoarthritis by quercetin-AKT1/IL6 and luteolin-VEGFA direct binding. Our findings have significant implication for our understanding of the molecular mechanism of action in the treatment of osteoarthritis and future development of better treatment of osteoarthritis.

Our results provide a scientific basis for further experimental studies to explore the molecular mechanism and application of Drynaria Fortunei and Cuscuta Chinensis in the treatment of osteoarthritis. We proposed that quercetin and luteolin may be used as potential active substances for the treatment of osteoarthritis.

Declarations

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

XH and HC perform bioinformatics analyses and wrote the draft. YW and XZ revised and edited the manuscript. KL, KH and HX supervised the project and revised the manuscript. All authors read and approved the final manuscript.

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Figures

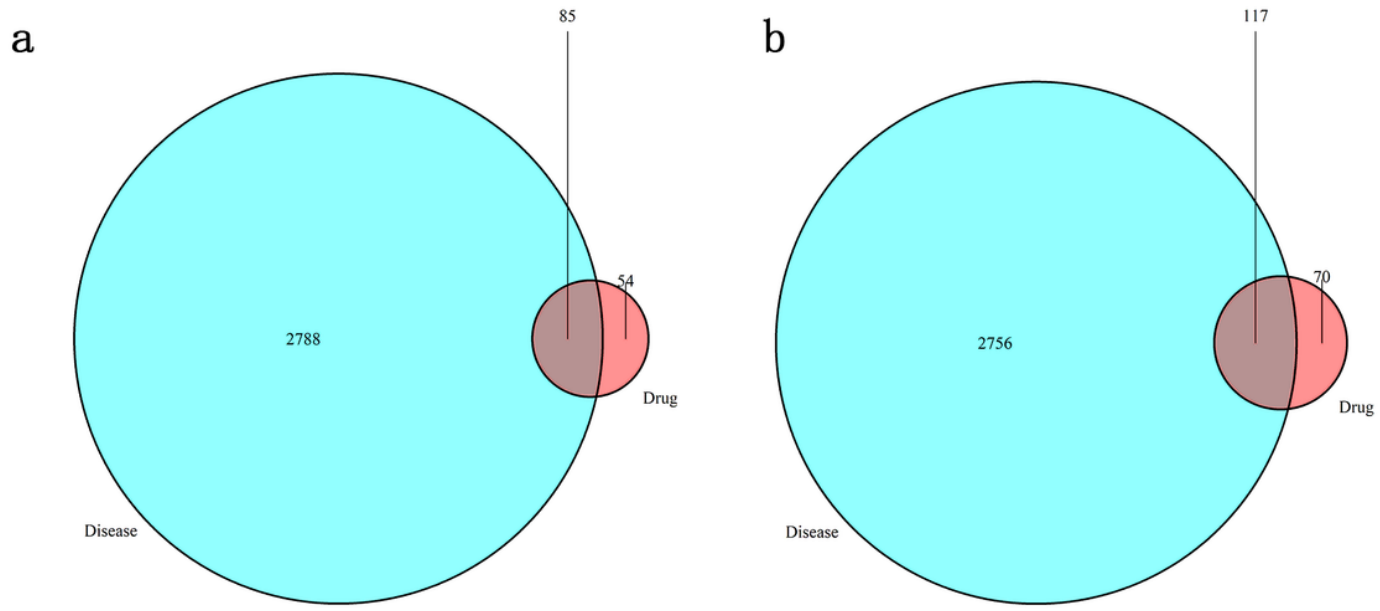

Figure 1

A Venn diagram of the 85 and 117 common target proteins of osteoarthritis and Drynaria Fortunei (a) / Cuscuta Chinensis targets (b).

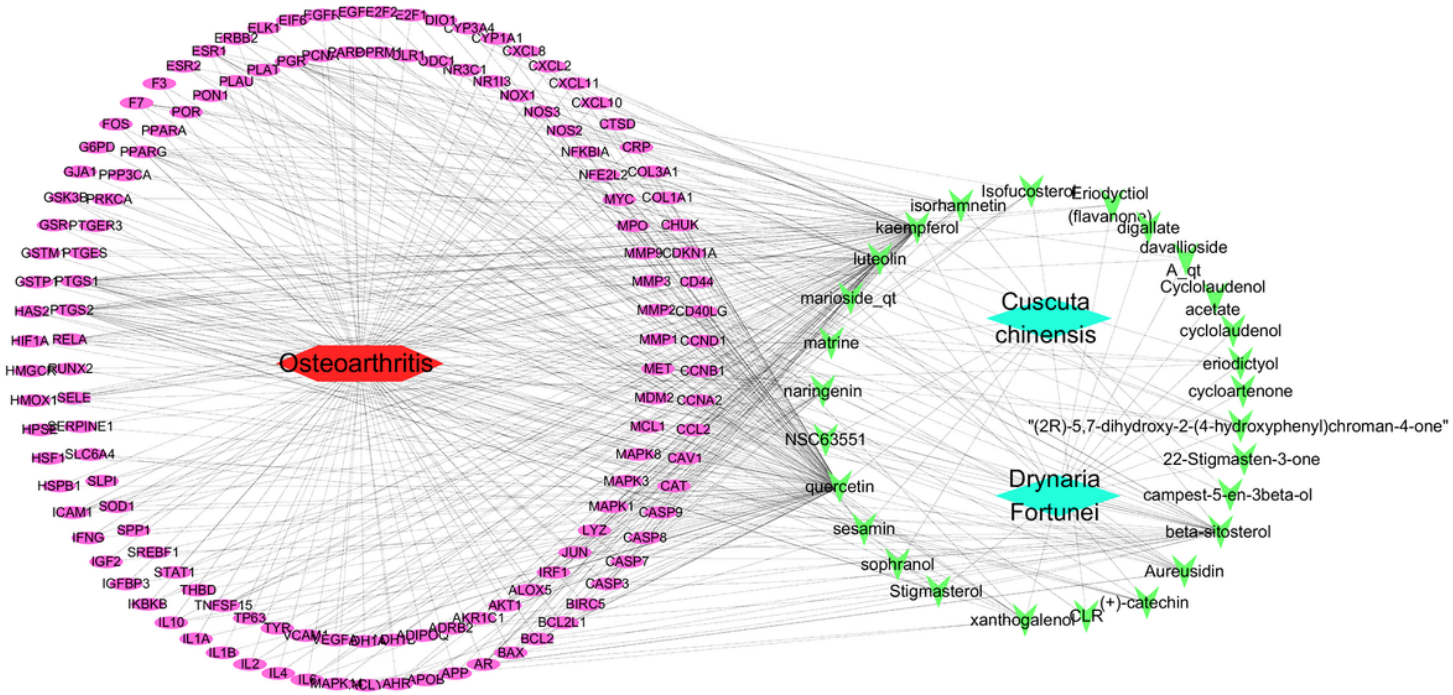

Figure 2

3 top active components were identified for osteoarthritis by pharmacological and PPI network analysis. The connectivity in the network represents the correlation of two molecules. Red represents osteoarthritis; sky blue represents TCM Drynaria Fortunei and Cuscuta Chinensis; green represents Drynaria Fortunei and Cuscuta Chinensis components; and purple represents the common targets of the TCM and osteoarthritis. PPI, protein-protein interaction; TCM, traditional Chinese medicine.

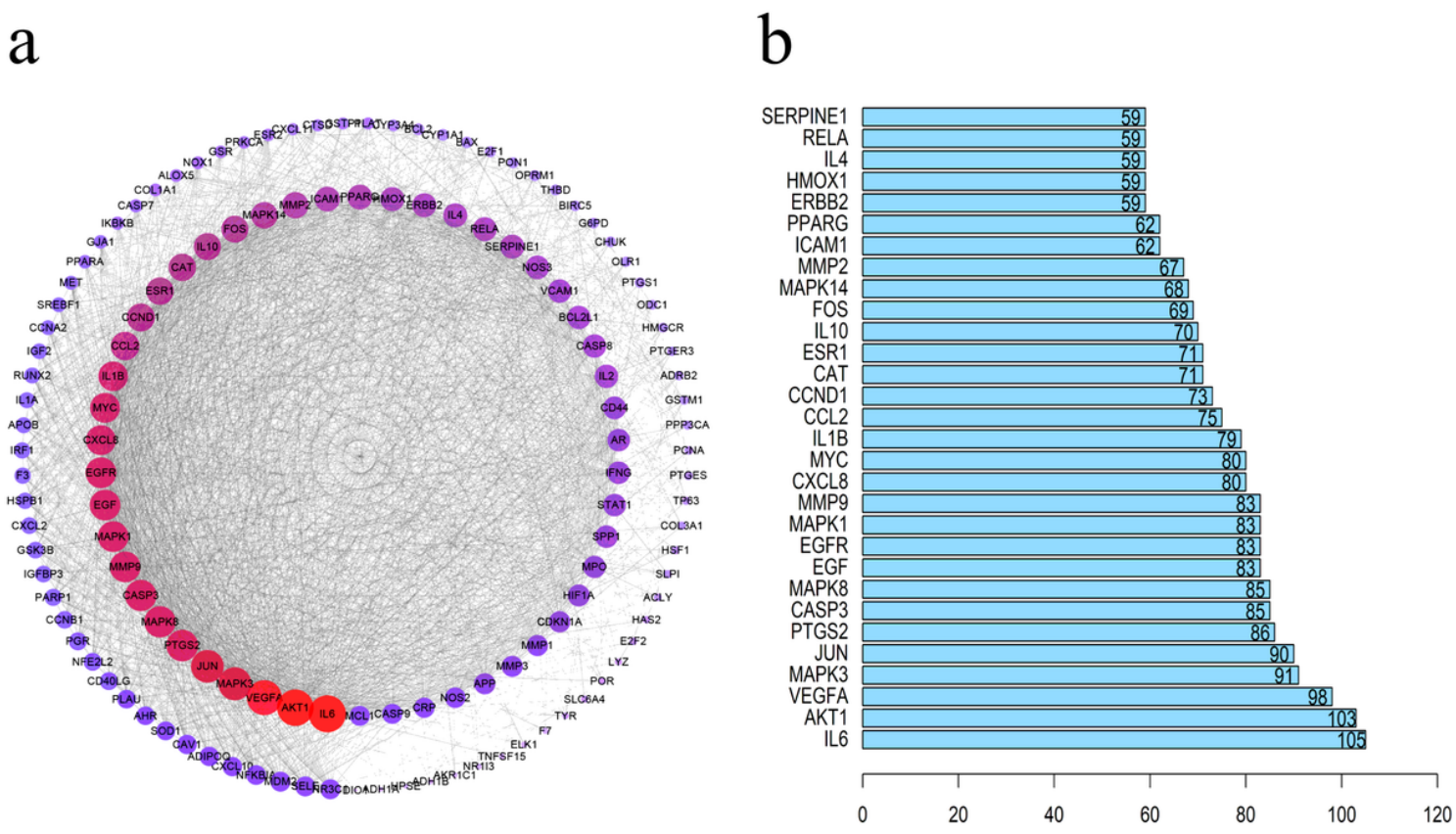

Figure 3

The 30 core common target proteins were identified by PPI network analysis. (a) The redder the color, the greater the number of adjacent nodes, the greater the probability of becoming a core gene. Where the node represents the protein, the line represents the interaction between proteins, the area of the node represents the degree value (number of adjacent nodes). The larger the node area, the redder the color, indicates the larger the degree value of the protein. (b) Barplot of the 30 TCM-osteoarthritis core targets. Left column: the name of the proteins; number: the number of adjacent nodes of the corresponding protein.

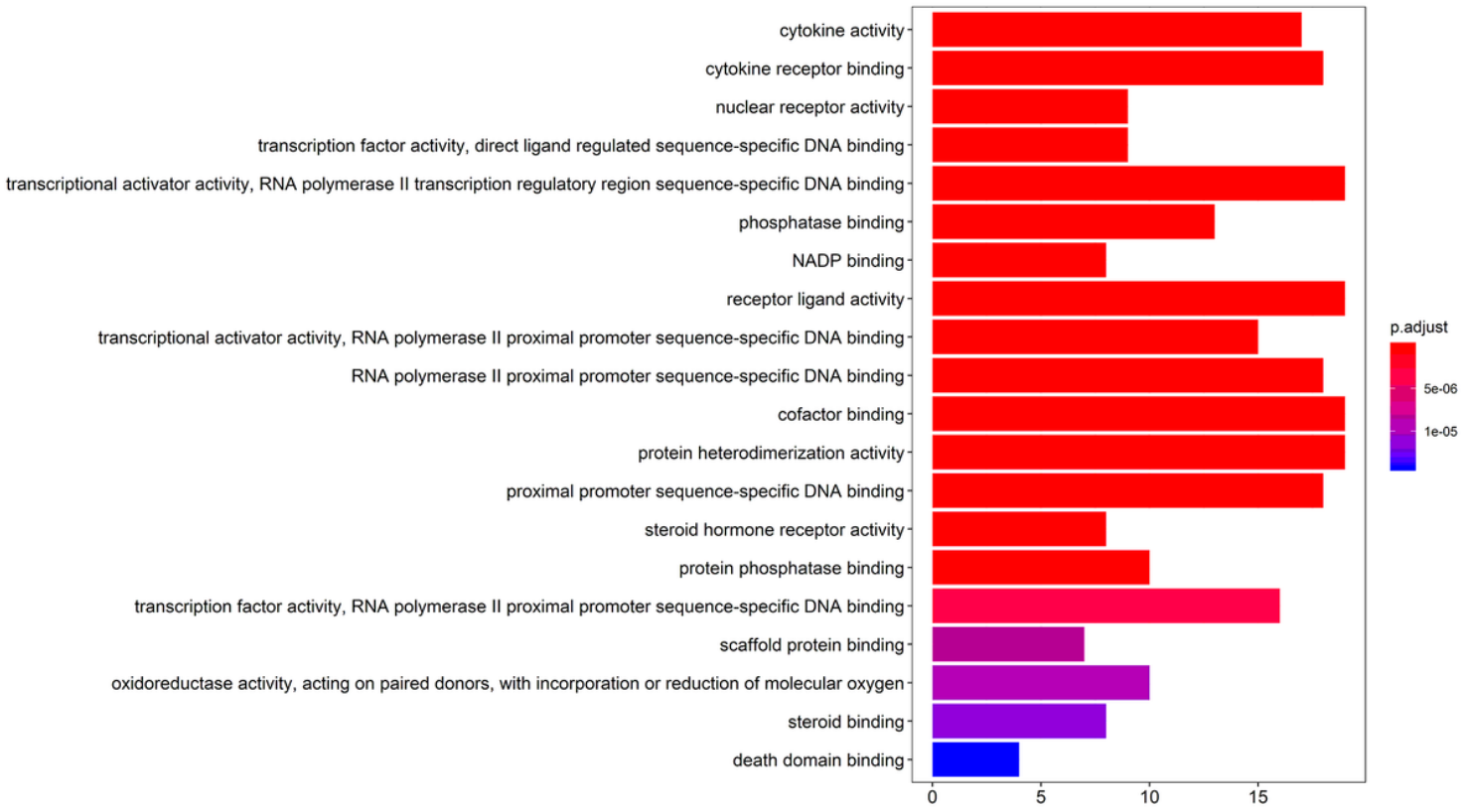

Figure 4

Dotplot for GO functional enrichment analysis. The letters on the left are the names of GO. The number is the number of genes enriched on GO. The bar graph represents the genes enriched on GO. P represents the significance of enrichment; the redder the color, the higher the enrichment degree, the corresponding smaller the P value, and P <0.05. GO: gene Ontology.

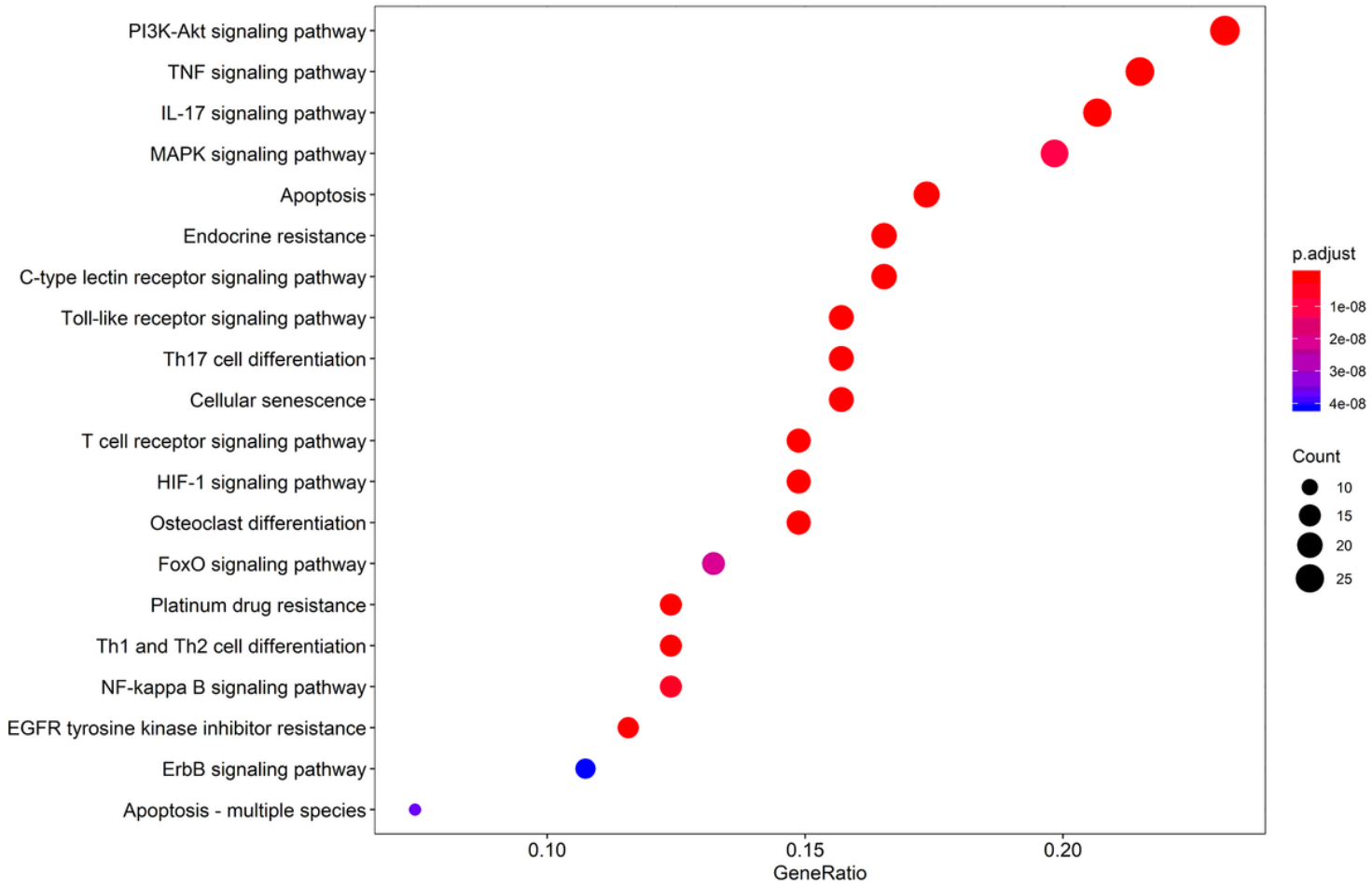

Figure 5

Barplot for KEGG pathway enrichment analysis. Left letters: KEGG names; horizontal axis number: the ratio of genes; circle size: number of enriched genes; color: represents P value, the redder the color, the higher the degree of enrichment, the smaller the corresponding P value, and P <0.05. KEGG, pathway enrichment analysis of a top module.

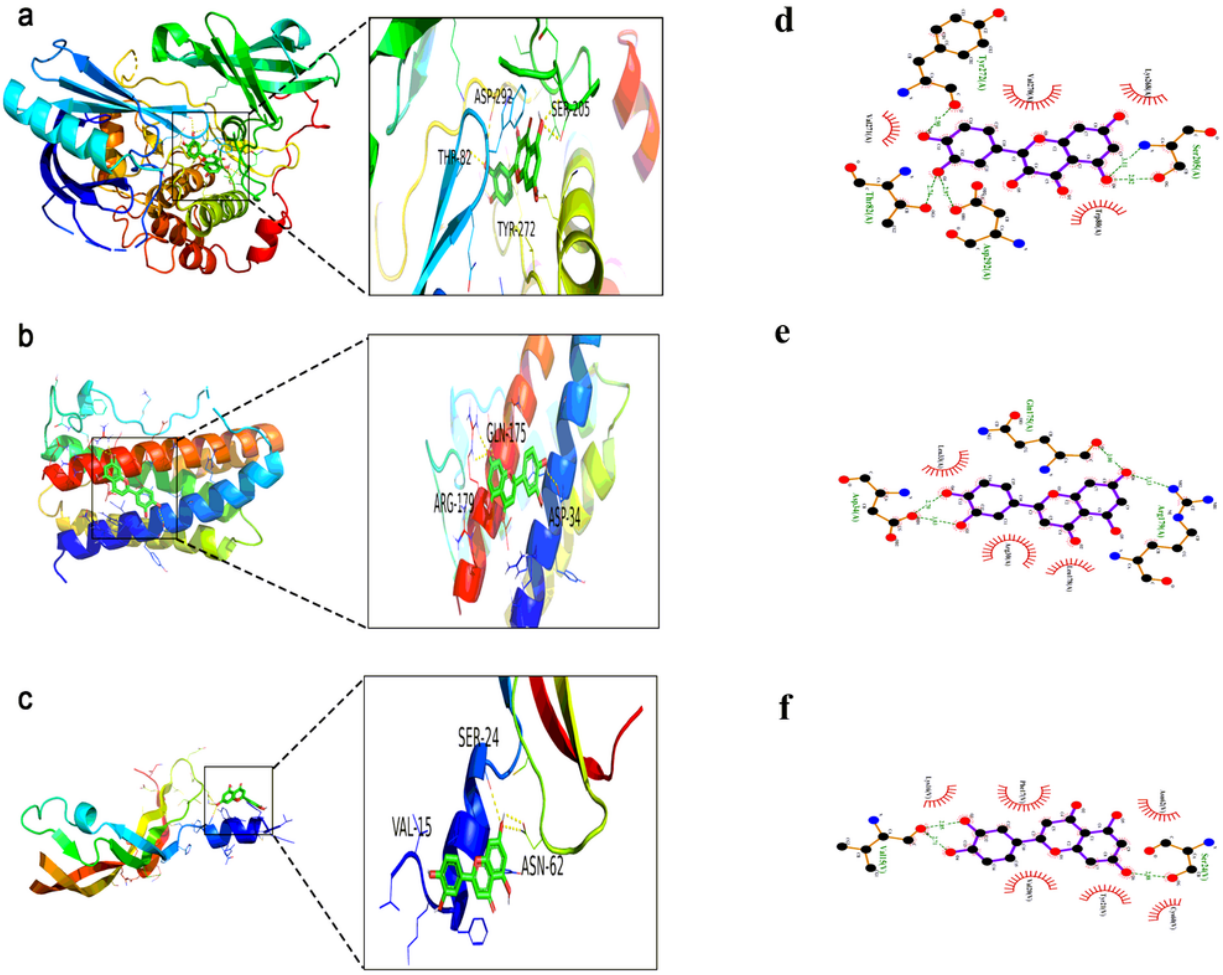

Figure 6

Structural binding modes of AKT1-quercetin (a), IL6-luteolin (b), and VEGFA-luteolin (c). Two-dimensional graphs of the interaction bind modes of AKT1-quercetin (d), IL6-luteolin (e), and VEGFA-luteolin (f). The interactions were mediated by hydrogen bonding and hydrophobic contact. The broken lines are the hydrogen bonds, and the value is the bond length. The arcs indicated hydrophobic contacts. Black dots represent carbon, red dots represent oxygen, and blue dots represent nitrogen. Black characters represent amino acids.