MEDS Clinical Medicine (2023)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2023.040405
ISSN 2616-1907 Vol. 4 Num. 4

Mechanism of "Huang Qi-Yin Yang Huo" on the Treatment of Osteoporosis by Active Components Based on Network Pharmacology and Molecular Docking

Changwen Zhou[1,a], Lixue Yang[1,b,*], Hongzhong Ma[2,c], Ce Liu[2,d]

[1]Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712046, China
[2]Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712000, China
[a]657152497@qq.com, [b]ylx18391076233@163.com, [c]1638948223@qq.com, [d]489635649@qq.com
**Corresponding author*

Keywords: Network pharmacology; Osteoporosis; Huang Qi; Yin Yang Huo; Molecular docking; Mechanism of action

Abstract: Objective: To investigate the effect of Huang Qi-Yin Yang Huo on osteoporosis (OP) by means of network pharmacology and molecular docking, in order to provide a basis for its basic research and clinical application. Methods: The active components and targets of the drug pair were obtained by means of TCMSP database and literature supplement. OMIM, GeneCards, DisGeNet, TTD and DrugBank databases were used to screen the targets related to osteoporosis, and Wayne tool was used to obtain the intersection targets. CytoScape 3.7.2 software was used to construct the "drug-ingredient-disease" network and screen core components according to node-degree values. Protein interaction network (PPI) was constructed with STRING 11.5, and core targets were screened according to node-degree values. Metascape was used for gene ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Gene and Genes (KEGG) pathway enrichment analysis, and the interactions between core components and core targets were verified by molecular docking. Results: 46 kinds of active components were identified by drug pair screening, and 128 intersection targets were identified. The target protein enrichment pathways included MAPK signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway and estrogen signaling pathway. The results of Molecular docking showed that the core components had good binding ability with the core target. Conclusion: Huang Qi-Yin Yang Huo may treat osteoporosis through multi-target and multi-pathway treatment, mainly regulating osteoblast differentiation, osteoclast inhibition, cell apoptosis and inflammation regulation, etc., providing a new idea and method for further research on the mechanism of osteoporosis in the future.

1. Introduction

Osteoporosis (OP) is a systemic skeletal disorder characterized by a reduction in bone strength, which increases the risk of fracture due to changes in both bone density and mass. Research

indicates that as of March 2020, the worldwide prevalence of osteoporosis in older adults is 21.7%, with the highest incidence observed among elderly individuals in Asia, reaching 24.3% [1]. This concerning situation demands our attention.

One of the pathological mechanisms of osteoporosis involves osteoclast stimulation and osteoblast inhibition, which leads to an imbalance between bone formation and resorption, ultimately resulting in decreased bone mass. To counteract this, three major classes of drugs are currently utilized in clinical practice: bone mineralization promoters, bone resorption inhibitors, and bone formation promoters. Continuous supplementation of calcium and vitamin D serves as the foundation of treatment, while bisphosphonates are recommended as the preferred drugs in several guidelines. However, a range of adverse reactions to bisphosphonates, including osteonecrosis of the jaw, musculoskeletal pain, esophageal cancer, and renal failure, have been reported in the 2011 Adverse Drug Reaction Information Bulletin [2]. Therefore, it is increasingly important to explore the use of traditional Chinese medicine and herbal remedies for the management of osteoporosis.

According to traditional Chinese medicine, osteoporosis is classified as "bone impotence", "bone paralysis" and "bone dryness", which is a chronic degenerative disease of the entire body caused by deficiency of kidney essence, bone dryness, reduction of marrow, and loss of nourishment of bone. The main treatment approach involves warming the liver and kidney. Qiu Yue et al. conducted an analysis of almost 20 years of literature on the treatment of osteoporosis and found that the Huang Qi-Yin Yang Huo pair had a confidence level of 0.63, indicating that when Huang Qi was used, Yin Yang Huo appeared 63% of the time and appeared together 49 times. The elevation was 1.29, suggesting that the Huang Qi-Yin Yang Huo pair showed a positive correlation and had significant discussion value [3]. To further clarify the mechanism of action of the two drugs in combination for the treatment of osteoporosis, this study employed network pharmacology and molecular docking to investigate the chemical composition, targets of action, and signaling pathways of the drugs at the molecular level, aiming to provide a theoretical basis for further development of Chinese medicine pharmacology for osteoporosis treatment.

2. Materials and Methods

2.1. Acquisition of active components in Huang Qi-Yin Yang Huo pair

The chemical constituents were retrieved from the TCMSP database [4] using "Huang Qi" and "Yin Yang Huo" as search keywords, and further filtered based on drug-like properties (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30%. Additional components of five herbs were incorporated based on literature [5, 6], leading to the final results.

2.2. Acquisition of active ingredient targets

The active components identified in "1.1" were imported into the TCMSP database for target prediction. Additionally, the UniProt database was utilized to standardize the drug target names, with "human" as the limited species and "reviewed" as the filter condition, to obtain the potential targets of the Huang Qi-Yin Yang Huo pair.

2.3. Obtaining potential targets for the Huang Qi-Yin Yang Huo pair in the treatment of osteoporosis

To ensure the data's comprehensiveness, the OMIM database [7] (Online Mendelian Inheritance in Man), GeneCards database (http://www.genecards.org), DisGeNet database [8] (https://www.disgenet.org), TTD database [9] (Therapeutic Target Database), and DrugBank

database [10] (https://go.drugbank.com/) were searched for the keyword "osteoporosis". Among these, the DrugBank database was constructed using clinical trial drug data, while OMIM and other databases were constructed based on literature research, each database having a different focus. Targets were integrated, and duplicate values were removed to obtain disease targets related to osteoporosis. The UniProt database was used to standardize the disease targets and exclude problems arising from different target name formats. The intersection of potential drug targets and disease targets was analyzed using the online Venn analysis tool, jvenn [11]. The resulting intersection targets represent the potential targets of Huang Qi-Yin Yang Huo pair for treating osteoporosis.

2.4. Constructing drug-component-target-disease networks and obtaining core components

The intersection targets from "1.3" were imported into CytoScape 3.7.2 to construct a "drug-component-target-disease" network. The "Network Analyzer" plug-in in CytoScape 3.7.2 was utilized to perform topological parameter analysis of the active components and disease targets. The degree value indicates the number of connections an ingredient has with the target, i.e., the number of targets the ingredient acts on. The top five components with the highest degree values were selected and identified as the core components for the drug to treat the disease.

2.5. Constructing PPI networks and acquiring core targets

The Protein-Protein Interaction (PPI) network consists of proteins that interact with each other to participate in various biological processes, such as biological signaling, gene expression regulation, energy and material metabolism, and cell cycle regulation. To systematically describe the role of target proteins at the biological system level, the intersection target data obtained from "1.3" were uploaded to the STRING 11.5 database [12], with the Organisms set as "Homo sapiens". The minimum required interaction score was set to 0.4, and the disconnected nodes in the network were hidden to obtain the network data, which was then imported into CytoScape 3.7.2 software for drawing the PPI network diagram and analyzing topological parameters. The higher the degree value, the more important the target is in the process of drug treatment. The top five targets with the highest degree values were selected as the core targets.

2.6. Enrichment Analysis of GO and KEGG Pathways

The intersecting targets obtained in "1.3" were subjected to enrichment analysis using the Metascape database. The screening criterion was set to "H. sapiens" for both the "Input as species" and "Analysis as species" panels. GO and KEGG pathway analysis were performed sequentially, and the results were visualized as bar graphs and bubble plots.

2.7. Molecular docking

To perform molecular docking, the 2D structures of the core components were obtained from the PubChem database [13] and optimized using Chem3D 17.0 software. The protein structures of the core targets were obtained from the PDB database [14] and saved in pdb format. The small molecule ligands and protein receptors were prepared using AutoDockTools-1.5.6 software [15], which involved removing the original ligands and water molecules from the crystal structure of receptors, adding nonpolar hydrogens, and assigning Gasteiger partial charges. The ligands were energy minimized and their atomic charges and atom types were assigned using the same software. The prepared data sets were then used for molecular docking and binding energy calculations using

AutoDock Vina 1.2.0 software [16], and the results were visualized for analysis.

3. Results

3.1. Acquisition of active components in Huang Qi-Yin Yang Huo pair

After exploring the TCMSP database and meticulously evaluating the parameters of OB≥30% and DL≥0.18, a total of 20 Huang Qi components and 23 Yin Yang Huo components were retrieved. Additionally, a further 5 Huang Qi components were identified through an extensive literature review. The consolidated dataset was then thoroughly scrutinized to remove any duplications, resulting in 2 common components, 23 unique components for Huang Qi, and 21 unique components for Yin Yang Huo, each assigned with a specific component number. Please refer to Table 1 for further details.

Table 1: Information on the main active components of the drug pair

Drug	Mol Id	Mol Name	OB	DL	Id
BOTH	MOL000098	quercetin	46.43	0.28	A
BOTH	MOL000422	kaempferol	41.88	0.24	B
HQ	MOL000211	Mairin	55.38	0.78	HQ1
HQ	MOL000239	Jaranol	50.83	0.29	HQ2
HQ	MOL000296	hederagenin	36.91	0.75	HQ3
HQ	MOL000354	isorhamnetin	49.6	0.31	HQ4
HQ	MOL000371	3,9-di-O-methylnissolin	53.74	0.48	HQ5
HQ	MOL000378	7-O-methylisomucronulatol	74.69	0.3	HQ6
HQ	MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92	HQ7
HQ	MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42	HQ8
HQ	MOL000387	Bifendate	31.1	0.67	HQ9
HQ	MOL000392	formononetin	69.67	0.21	HQ10
HQ	MOL000430	betaine	40.92	0.01	HQ11
HQ	MOL000433	FA	68.96	0.71	HQ12
HQ	MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26	HQ13
HQ	MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62	HQ14
HQ	MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48	HQ15
HQ	MOL000401	astragalosideI	46.79	0.11	HQ16
HQ	MOL000403	astragalosideII	46.06	0.13	HQ17
HQ	MOL000405	astragalosideIII	31.83	0.1	HQ18
HQ	MOL000407	astragalosideIV	22.5	0.15	HQ19
HQ	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-cyclopenta[a]phenanthren-3-ol	36.23	0.78	HQ20
HQ	MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69	HQ21
HQ	MOL000398	isoflavanone	109.99	0.3	HQ22
HQ	MOL000417	Calycosin	47.75	0.24	HQ23
YYH	MOL000006	luteolin	36.16	0.25	YYH1
YYH	MOL000359	sitosterol	36.91	0.75	YYH2
YYH	MOL000622	Magnograndiolide	63.71	0.19	YYH3
YYH	MOL001510	24-epicampesterol	37.58	0.71	YYH4
YYH	MOL001645	Linoleyl acetate	42.1	0.2	YYH5

YYH	MOL001792	DFV	32.76	0.18	YYH6
YYH	MOL003044	Chryseriol	35.85	0.27	YYH7
YYH	MOL003542	8-Isopentenyl-kaempferol	38.04	0.39	YYH8
YYH	MOL004373	Anhydroicaritin	45.41	0.44	YYH9
YYH	MOL004380	C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	39.14	0.49	YYH10
YYH	MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	YYH11
YYH	MOL004367	olivil	62.23	0.41	YYH12
YYH	MOL004382	Yinyanghuo A	56.96	0.77	YYH13
YYH	MOL004384	Yinyanghuo C	45.67	0.5	YYH14
YYH	MOL004386	Yinyanghuo E	51.63	0.55	YYH15
YYH	MOL004388	6-hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-dioxo-2,3,4,8-tetrahydro-1H-isochromeno[3,4-h]isoquinolin-2-ium	60.64	0.66	YYH16
YYH	MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	48.54	0.25	YYH17
YYH	MOL004394	Anhydroicaritin-3-O-alpha-L-rhamnoside	41.58	0.61	YYH18
YYH	MOL004396	1,2-bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol	52.31	0.22	YYH19
YYH	MOL004425	Icariin	41.58	0.61	YYH20
YYH	MOL004427	Icariside A7	31.91	0.86	YYH21

3.2. Prediction of Component Targets and Disease Targets

The TCMSP database was queried to acquire 242 targets for the components, while the OMIM, GeneCards, DisGeNet, TTD, and DrugBank databases were searched to gather 2240 targets related to osteoporosis. After consolidating and removing any duplicates among the disease targets, the UniPort database was utilized to standardize the component targets and disease targets into Gene Symbol, thereby resolving issues stemming from dissimilar target name formats. Employing the jvenn tool, the intersection of these two datasets yielded 128 prospective targets for the treatment of osteoporosis using the Huang Qi-Yin Yang Huo pair, as depicted in Figure 1.

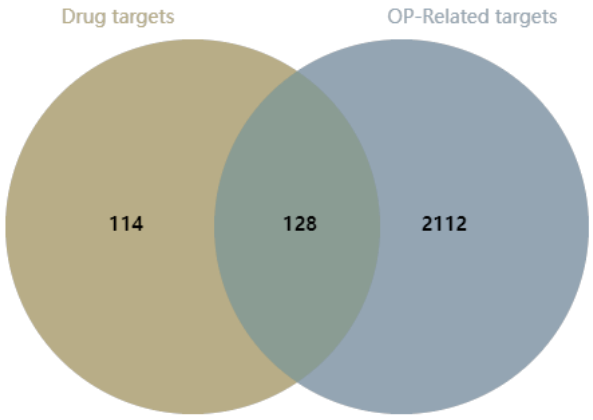


Figure 1: Venn diagram of drug targets and disease targets

3.3. Drug-component-target-disease network construction and core component acquisition

After excluding the active components that could not be matched to reliable targets, the remaining 23 active components and 128 intersecting targets were fed into Cytoscape software to

construct a comprehensive drug-component-target-disease network, as depicted in Figure 2. This network consists of 154 nodes and 377 edges, with different nodes represented by different icons and colors. Specifically, the yellow and green icons represent Huang Qi and Yin Yang Huo, respectively, and their main active components; the two-color icon stands for the shared ingredient between the two herbs; the blue icon signifies the common target between the drug and disease; the red icon denotes the disease. The connecting lines denote the interaction between the drug, ingredient, target, and disease. The size and transparency of each node reflect the degree value, with larger and clearer nodes representing a more prominent position in the network. The findings indicate that the active components of the Huang Qi-Yin Yang Huo drug pair can simultaneously modulate multiple targets of action, with quercetin (A), kaempferol (B), luteolin (YYH1), isorhamnetin (HQ4), and Jaranol (HQ2) exhibiting the highest degree values in the network.

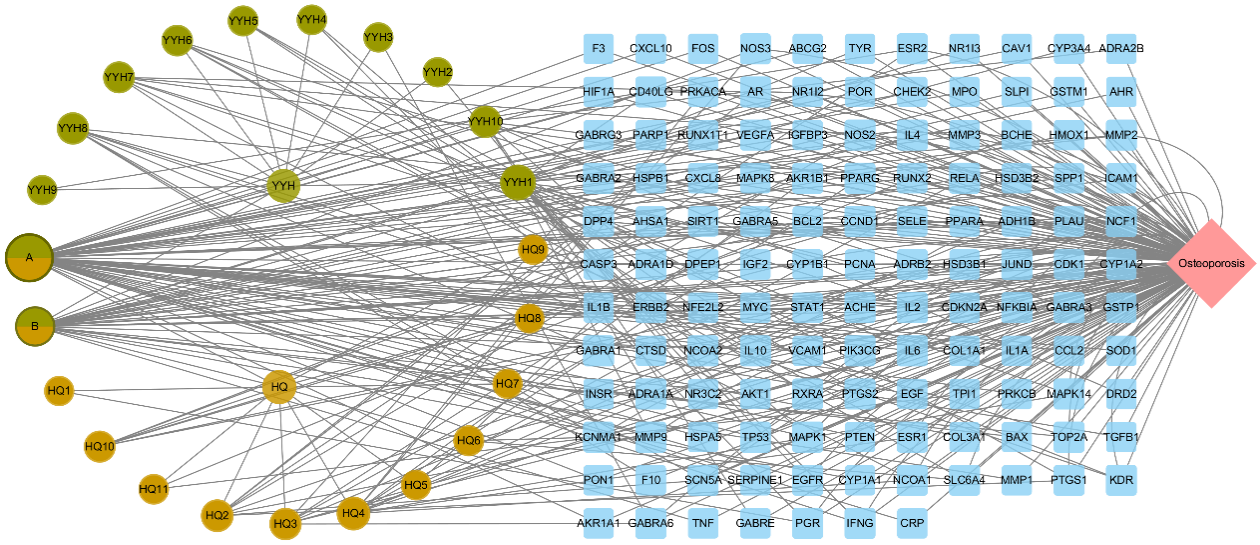


Figure 2: Drug-component-target-disease interaction network

3.4. Constructing PPI networks and acquiring core targets

After importing the intersection targets into the STRING database, PPI data was obtained and imported into Cytoscape 3.7.2 software to reconstruct the PPI network (Figure 3). The network consisted of 128 nodes, representing functional proteins, and 2194 edges, representing interaction relationships. Node degree value was used as an evaluation criterion to rank the nodes, where larger nodes with darker colors indicate more targets have interaction relationships with the node, making it more important in the network. The top 5 core targets with degree values were AKT1, TP53, TNF, IL6, and VEGFA.

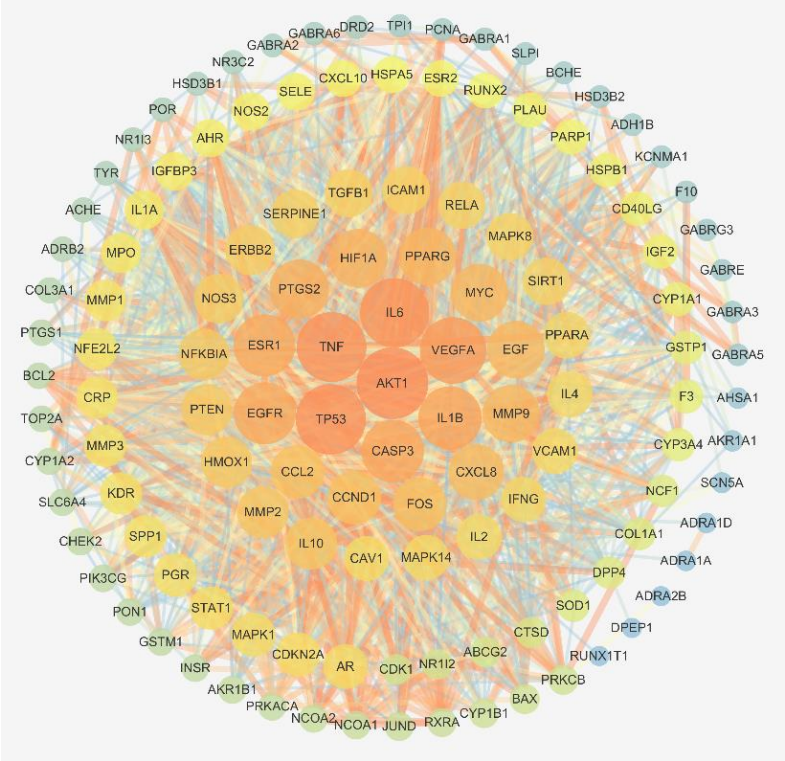
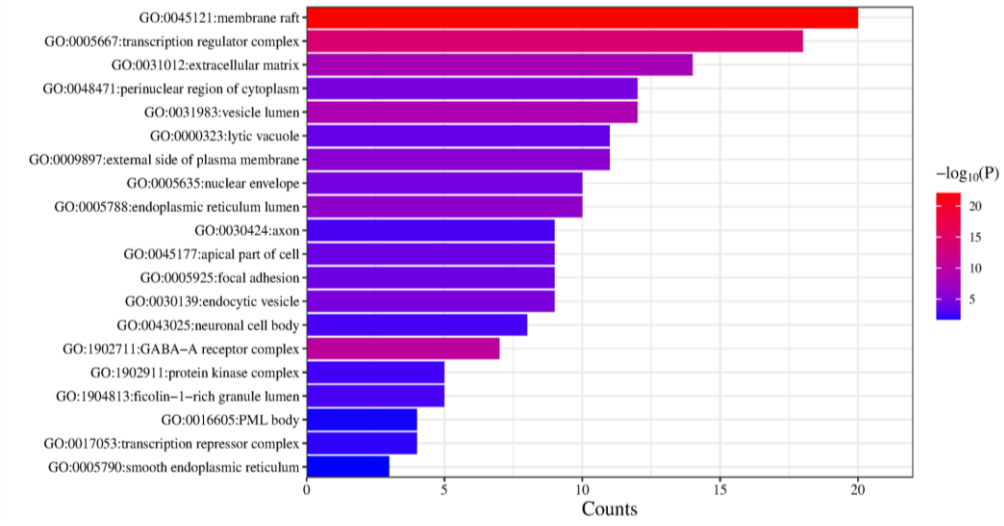
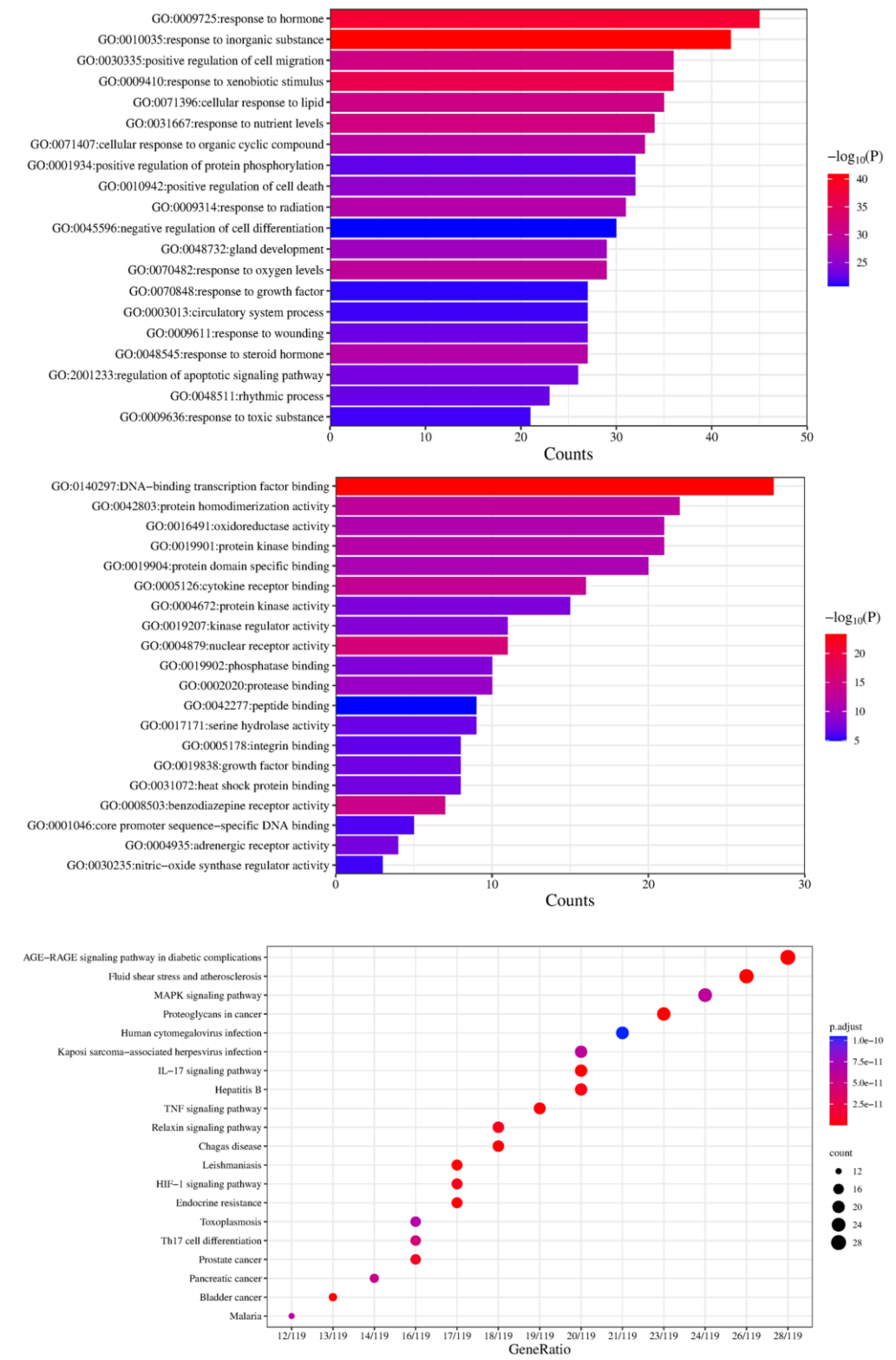


Figure 3: PPI network of Huang Qi-Yin Yang Huo pair on targets related to the treatment of osteoporosis

3.5. Enrichment Analysis of GO and KEGG Pathways

As per the analysis conducted using the Metascape database, the bioinformatics analysis of targets related to the treatment of osteoporosis with the Huang Qi-Yin Yang Huo pair is depicted in Figure 4. The results of the GO-BP analysis reveal that the primary biological processes involved in treating osteoporosis with the drug pair encompass response to inorganic substance, response to hormone, response to xenobiotic stimulus, and response to nutrient levels. On the other hand, the KEGG analysis reveals the top 20 signaling pathways for treating osteoporosis, wherein the critical pathways include MAPK signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, Estrogen signaling pathway, etc.





Note: Top left: GO-MF analysis; top right: GO-BP analysis; bottom left: GO-CC analysis; bottom right: KEGG analysis

Figure 4: Enrichment analysis of potential targets of Huang Qi-Yin Yang Huo pair for the treatment of osteoporosis

3.6. Molecular docking

To further elucidate the binding efficacy between the targets and components of the Huang Qi-Yin Yang Huo pair for the treatment of osteoporosis, the core components that were screened in section "2.3" were selected for molecular docking with the core targets that were screened in section "2.4". Ultimately, 25 sets of receptor-ligand docking outcomes were obtained. If the affinity is less than zero, it indicates that the molecules possess binding activity, and the lower the value, the more robust the binding ability, as depicted in Table 2. The docking findings can offer screening strategies for further research and experimental design, and several of the docking demonstrations are depicted in Figure 5.

Table 2: Affinity of core components and core target molecules (kcal/mol)

Gene	quercetin	kaempferol	luteolin	isorhamnetin	Jaranol
AKT1	-6.8	-6.9	-6.7	-6.8	-6.8
TP53	-7.2	-7.1	-7.5	-7.4	-6.7
TNF	-10.2	-10.2	-10.7	-10.3	-9.8
IL6	-6.9	-6.8	-7.2	-7.1	-6.7
VEGFA	-6.9	-6.8	-7	-6.8	-6.6

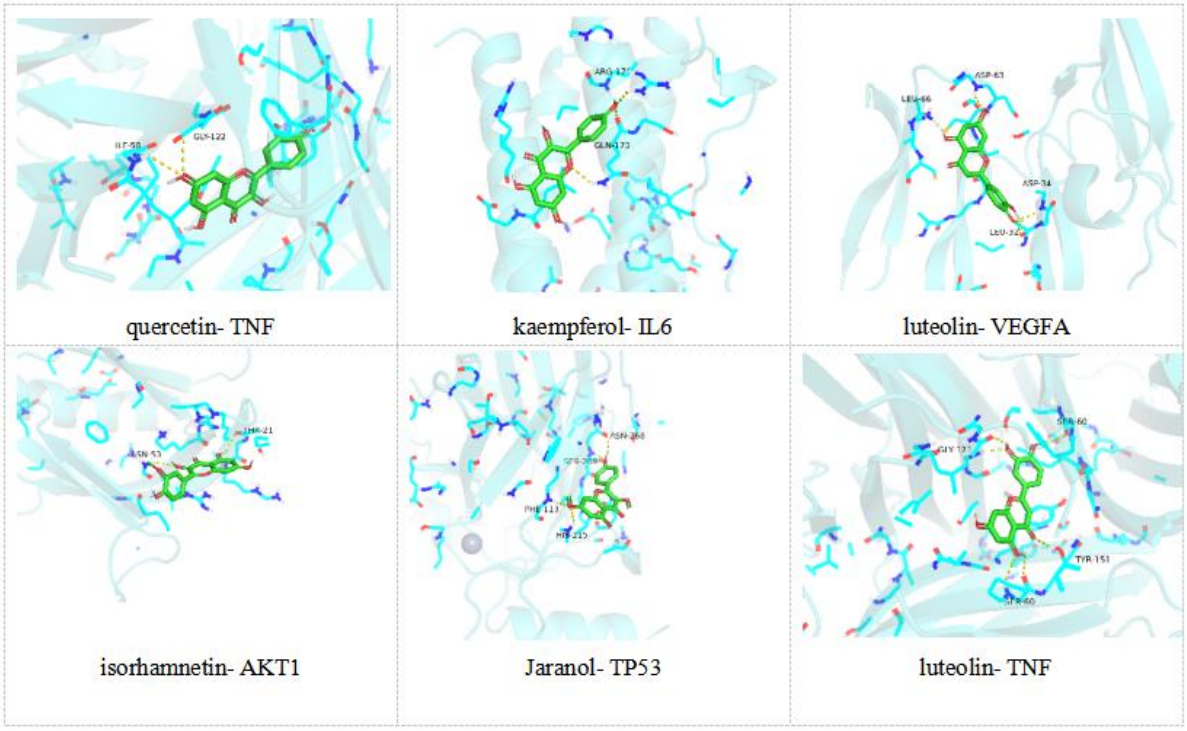


Figure 5: Molecular docking of core components and targets of Huang Qi-Yin Yang Huo pair

4. Discussion

Osteoporosis is a widespread ailment among orthopedic patients. As evidenced by numerous prior epidemiological studies, age is an autonomous risk factor for the onset of osteoporosis [17], and its prevalence has been progressively escalating with the aging population. Concurrently, the negative effects of bisphosphonates, commonly used in Western medicine, have been gradually exposed, thereby augmenting the demand for new, safer and more efficient therapeutic alternatives. Chinese medicine, being safer and more potent, offers a viable alternative. Although Chinese

medicine lacks a distinct term for "osteoporosis," it employs terms such as "bone impotence," "bone paralysis," "bone dryness," etc. to describe the ailment. The therapy of osteoporosis, in Chinese medicine, involves nourishing the liver, kidney, spleen, and stomach in the case of deficiency and invigorating qi and blood to reduce pain in the case of stagnation, and has yielded good results when applied in a bidirectional manner to address both causes and symptoms. Thus, this study, through a combination of literature analysis, selected "Huang Qi-Yin Yang Huo" as the research subject. This research analyzed the active ingredients, potential targets, modes of action, and pathways of the drug for treating diseases based on network pharmacology and bioinformatics and performed preliminary validation using molecular docking.

4.1. Components

Based on the analysis of "2.3", the top five fundamental components are quercetin, kaempferol, luteolin, isorhamnetin, and Jaranol. Among these, quercetin, as a common component of Huang Qi and Yin Yang Huo, displays a wide range of biological activities, making it particularly noteworthy for disease prevention and health promotion. Studies have shown that quercetin protects bones by inhibiting RANKL-mediated osteoclast development and osteoblast apoptosis, reducing oxidative stress and inflammation, and promoting osteogenesis, angiogenesis, and antioxidant expression, as well as adipocyte apoptosis and osteoclast apoptosis. The potential underlying mechanisms involved are Wnt, NF-κB, Nrf2, SMAD dependence, and regulation of intrinsic and extrinsic apoptotic pathways [18].

Kaempferol, another typical component of Huang Qi and Yin Yang Huo, displays a bone-building effect and exhibits potential effects in the prevention and treatment of osteoporosis. Studies have found that kaempferol protects bones by inhibiting the inflammatory response and oxidative stress, autophagy of osteoclasts, and apoptosis of osteoblasts, and activating the autophagy of osteoblasts [19].

Luteolin is a polyphenolic compound from Epimedium that prevents bone loss in animal models of ovariectomy-induced osteoporosis. Kim et al. found that luteolin can prevent bone loss caused by postmenopausal osteoporosis by inhibiting osteoclast differentiation and function [20].

Isorhamnetin, as a symbiotic flavonoid of quercetin and the metabolized form of quercetin in vivo, regulates the function of osteoblasts and osteoclasts by regulating the RANKL/RANK/OPG signaling pathway and improves the bone microtrophy caused by ovariectomized rats. Additionally, isorhamnetin can activate the p38 signaling pathway to promote osteogenic differentiation of bone marrow mesenchymal stem cells, and prevent and treat osteoporosis from two aspects [21].

However, Jaranol's research is relatively shallow, and its biological activity remains poorly characterized, leading it to be neglected in previous studies. Although network pharmacology relies solely on the existing database and experimental data for network modeling, there is a certain probability of false positives and false negatives, but the results of molecular docking demonstrate that Jaranol has robust binding activity with the core target, indicating that the future experimental design should consider the relevant mechanisms and effects of Jaranol on osteoporosis intervention.

4.2. Targets

Through the analysis of PPI network and "Network Analyzer", the primary targets of Huang Qi and Yin Yang Huo for treating osteoporosis are AKT1, TP53, TNF, IL6, and VEGFA. AKT1 is a critical regulator of osteoblasts and osteoclasts that promotes the differentiation and storage of these cells to maintain bone mass and turnover. Mukherjee's research shows that a deficiency of AKT1, but not AKT2, can lead to a decrease in bone mineral density, cortical bone thickness, and trabecular bone thickness in the whole body. AKT1 also affects cell proliferation and osteoclast

differentiation [22]. TP53, or P53, is a tumor suppressor that plays an important role in inhibiting bone and soft tissue sarcoma. However, research by Yu et al. suggests that P53 has a potential role in the development or progression of osteoporosis, as the level of P53 protein in osteoporosis patients is higher than that in healthy controls. Down-regulating P53 can suppress the characteristic indicators of osteoporosis [23]. TNF-α and IL-6 are proinflammatory cytokines that play crucial roles in immune responses and bone metabolism. TNF-α inhibits the activity of osteoblasts during the differentiation stage and stimulates the proliferation and differentiation of osteoclasts. IL-6 mediates the actions of both osteoblasts and osteoclasts and can also regulate the activity of osteocytes. Both TNF-α and IL-6 are important pathogenic factors of postmenopausal osteoporosis and have considerable potential in the clinical treatment of osteoporosis [24]. VEGFA is a promoter of perichondral vascular and osteoblast differentiation in early bone development and plays a vital role in endochondral bone formation. The deletion of the VEGFA gene condition results in thinner bones, reduced bone mineralization, and lack of secondary ossification centers in mice, according to Duan et al.'s findings [25].

4.3. Enrichment Analysis of GO and KEGG Pathways

The KEGG analysis is primarily responsible for clarifying the pathway analysis of drug components attached to the target for function. The results demonstrate that the signaling pathways involved in the core target of Huang Qi and Yin Yang Huo in the treatment of osteoporosis include the MAPK signaling pathway, PI3K-Akt signaling pathway, Estrogen signaling pathway, HIF-1 signaling pathway, JAK-STAT signaling pathway, Wnt signaling pathway, Hedgehog signaling pathway, NF-kappa B signaling pathway, GABAergic synapse, and cAMP signaling pathway. Among these, the significantly stronger MAPK signaling pathway is an important way to regulate the growth and differentiation of osteoblasts. The sub-pathways included in the MAPK pathway control osteoblasts from the expression of osteoblasts, the differentiation of osteoblasts, and the later stage of differentiation. After activating the MAPK pathway, it promotes the differentiation and proliferation of osteoblasts. Compared to the enhanced activity of osteoclasts, the activity of osteoblasts is similar to or stronger than that of osteoclasts, thereby preventing osteoporosis [26, 27].

Peng et al. [28] demonstrated that after deleting AKT1 and AKT2 in the mouse gene, the mice showed obvious symptoms of osteopenia. When the inhibitor PTEN was deficient, the AKT signaling pathway was significantly enhanced, and the bone mass increased, suggesting the role of osteoblasts associated with PI3K-Akt, AKT, and its target genes, which when activated, promote osteoblast differentiation and mineralization. Osteoporosis is one of the metabolic bone diseases characterized by bone loss. Both osteogenesis reduction and osteoclastosis lead to bone loss. Most of the existing pathways start with the intervention of osteoblast differentiation to solve bone loss. The Estrogen signaling pathway reversely inhibits the differentiation of osteoclasts and intervenes in the bone resorption of osteoclasts, thereby preventing bone loss and osteoporosis [29].

5. Conclusion

This study has systematically analyzed and initially validated the mechanism of the Huang Qi-Yin Yang Huo pair in treating osteoporosis through network pharmacology and molecular docking research methods. With the rise of diseases caused by population aging, the analysis and implementation of characteristic medications serve as a crucial preventive measure. The study hopes that the findings can offer fresh insights for further research on the compatibility of osteoporosis drugs.

References

[1] Salari Nader, Ghasemi Hooman, Mohammadi Loghman, Behzadi Mohammad hasan, Rabieenia Elham, Shohaimi Shamarina, Mohammadi Masoud. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis [J]. Journal of Orthopaedic Surgery and Research,2021,16(1).

[2] Zhu Xiaoyu, Zhang Weiguang, Zhao Zhi-gang. Research status of drug therapy for osteoporosis at home and abroad [J]. The Chinese Journal of Clinical Pharmacology, 2020, 36(05): 588-92.

[3] Qiu Yue, Yu Rong, Xiong Tao, Wu Wenjie. Study on the medication regularity of traditional Chinese medicine in treatment of primary osteoporosis [J]. Chinese Journal of Osteoporosis, 2022, 28(01): 75-9.

[4] Jinlong Ru, Peng Li, Jinan Wang, Wei Zhou, Bohui Li, Chao Huang, Pidong Li, Zihu Guo, Weiyang Tao, Yinfeng Yang, Xue Xu, Yan Li, Yonghua Wang, Ling Yang. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines [J]. J. Cheminformatics,2014,6(1).

[5] Yang Liu, Zhang Wangning, Liu Yuetao, Li Aiping, Qin Xuemei. Mechanistic analysis of Astragali Radix in treatment of nephrotic syndrome using network pharmacology [J]. Chinese Traditional and Herbal Drugs, 2019, 50(08): 1828-37.

[6] Song Jianbo, Liao Hui, Li Yuanping. Mechanism exploration of Astragali Radix in treatment of diabetic nephropathy based on network pharmacology [J]. Chinese Traditional and Herbal Drugs, 2020, 51(11): 2988-96.

[7] Amberger Joanna S, et al. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders [J]. Nucleic acids research,2015,43(Database issue).

[8] Janet P , Àlex B , Núria Q , et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants [J]. Nucleic acids research,2017,45(D1):D833-D839.

[9] Zhou Ying, et al. Therapeutic target database update 2022: facilitating drug discovery with enriched comparative data of targeted agents [J]. Nucleic acids research,2021,50(D1):D1398-D1407.

[10] Wishart David S, et al. DrugBank 5.0: a major update to the DrugBank database for 2018 [J]. Nucleic acids research,2018,46(D1):D1074-D1082.

[11] Bardou Philippe, et al. jvenn: an interactive Venn diagram viewer [J]. BMC bioinformatics,2014,15(1):293.

[12] Szklarczyk Damian, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets [J]. Nucleic acids research,2019,47(D1):D607-D613.

[13] Sunghwan K , Jie C , Tiejun C , et al. PubChem in 2021: new data content and improved web interfaces [J]. Nucleic acids research, 2021, 49(D1): D1388-D1395.

[14] BURLEY S K, 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 [J]. Nucleic acids research, 2021, 49(D1): D437-D451.

[15] Zhang Yuqi, et al. AutoDock CrankPep: combining folding and docking to predict protein-peptide complexes [J]. Bioinformatics (Oxford, England), 2019, 35(24): 5121-5127.

[16] Eberhardt Jerome, et al. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings [J]. Journal of chemical information and modeling, 2021, 61(8): 3891-3898.

[17] Zhao Jingjing, et al. Prevalence and influencing factors of osteoporosis among adults in Hebei province [J]. Chinese Journal of Public Health, 2022, 38(06): 749-751.

[18] Wong Sok Kuan, et al. Quercetin as an Agent for Protecting the Bone: A Review of the Current Evidence [J]. International journal of molecular sciences, 2020, 21(17).

[19] Wong Sok Kuan, et al. The Osteoprotective Effects Of Kaempferol: The Evidence From In Vivo And In Vitro Studies [J]. Drug design, development and therapy, 2019, 13: 3497-514.

[20] KIM T H, JUNG J W, HA B G, et al. The effects of luteolin on osteoclast differentiation, function in vitro and ovariectomy-induced bone loss [J]. The Journal of nutritional biochemistry, 2011, 22(1): 8-15.

[21] Fan Jun, Cao Liping. Isorhamnetin activates p38 signaling to promote osteogenic differentiation of murine mesenchymal stem cells [J]. Chinese Journal of Joint Surgery(Electronic Edition), 2021, 15(04): 432-7.

[22] MUKHERJEE A, LARSON E A, KLEIN R F, et al. Distinct actions of akt1 on skeletal architecture and function [J]. PloS one, 2014, 9(3): e93040.

[23] Yu Tao, et al. p53 plays a central role in the development of osteoporosis [J]. Aging, 2020, 12(11): 10473-87.

[24] WANG T, HE C. TNF-α and IL-6: The Link between Immune and Bone System [J]. Current drug targets, 2020, 21(3): 213-27.

[25] DUAN X, MURATA Y, LIU Y, et al. Vegfa regulates perichondrial vascularity and osteoblast differentiation in bone development [J]. Development (Cambridge, England), 2015, 142(11): 1984-91.

[26] Xiao Yaping, et al. Review for treatment effect and signaling pathway regulation of kidney-tonifying traditional Chinese medicine on osteoporosis [J]. China Journal of Chinese Materia Medica, 2018, 43(01): 21-30.

[27] Li ihuo. The study on psoralen stimulating osteoblast proliferation by activating 1VF-kB-MAPK signaling [D]. Guangzhou University of Chinese Medicine, 2016.
[28] PENG X D, XU P Z, CHEN M L, et al. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2 [J]. Genes & development, 2003, 17(11): 1352-65.
[29] NAKAMURA T, IMAI Y, MATSUMOTO T, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts [J]. Cell, 2007, 130(5): 811-23.