International Journal of Pharmacology

ISSN 1811-7775

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2025.475.488

Research Article
Exploring the Mechanism of Action of Ru-Pi-Xiao on Treating Mammary Gland Hyperplasia Based on Network Pharmacology and Molecular Docking Technology

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Abstract

Background and Objective: Ru-Pi-Xiao is a classic Chinese medicine prescription, which has a remarkable effect in treating mammary gland hyperplasia. However, the primary constituents and underlying mechanisms of Ru-Pi-Xiao have yet to be fully elucidated. This study aims to provide an initial exploration of the potential mechanisms by which Ru-Pi-Xiao exerts its therapeutic effects on MGH through the application of network pharmacology and molecular docking techniques. **Materials and Methods:** Relevant targets of active ingredients and diseases were carried out through TCMSP, Gene Cards, DisGeNET and NCBI databases. Drug targets and disease targets were taken to make Venn diagrams, PPI network diagrams were made in the STRING database with shared targets, Core targets and compound drug-active ingredient-target network diagrams were drawn through Cytoscape software. The Metascape platform was used for GO enrichment analysis and KEGG analysis. Molecular docking was performed with AutoDockTools. **Results:** About 110 active ingredients and 147 intersecting genes were screened. The main biological processes were response to peptide, enzyme-linked receptor protein signaling pathway, protein phosphorylation, gland development and positive regulation of transferase activity. "Proteoglycans in cancer", "JAK-STAT signaling pathway", "Calcium signaling pathway" and other pathways were involved in the treatment. The core targets were "TP53", "SRC", "STAT3", "AKT1", "PIK3CA" and so on. Molecular docking revealed good binding ability between the active compounds and screened targets. Among them, Quercetin has good binding activity with TP53 and AKT1 and 6-Hydroxykaempferol has good binding activity with SRC and PIK3CA. **Conclusion:** The Ru-Pi-Xiao may exert its therapeutic effects on MGH through multi-components, multi-targets and multi-pathways.

Key words: Network pharmacology, Ru-Pi-Xiao, mammary gland hyperplasia (MGH), molecular docking

Citation: Pan, M., N. Jia, Z. Li, Q. Zhang, L. Sun and X. Chu, 2025. Exploring the mechanism of action of Ru-Pi-Xiao on treating mammary gland hyperplasia based on network pharmacology and molecular docking technology. Int. J. Pharmacol., 21: 475-488.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mammary gland hyperplasia (MGH) also known as "Ru-Pi" in Chinese, is a non-inflammatory and non-neoplastic lesion, which is a structural disorder of the mammary glands due to various degrees of hyperplasia and dysplasia of the mammary parenchyma and stroma[1]. Research shows that MGH is the highest incidence of female breast disease, about 75% of women have some degree of MGH, with the most frequent in women aged 25-45 years old[2]. Furthermore, the possibility of this disease developing into breast cancer is very high. Therefore, the treatment of this disease has become a hot spot of research. Treating MGH in modern medicine can usually be divided into two ways: Traditional Chinese Medicine (TCM) and Western medicine. Western medicine mostly adopts hormone drugs or surgery. However, taking hormone drugs will bring many serious side effects, such as menstrual disorders, nausea and vomiting, irritability, etc. and many complications will also occur in long-term use[3,4]. On the other hand, surgery will bring more pain to patients and have a high recurrence rate.

With the deepening of the research on the effect of MGH treatment by TCM[5-8], the advantages of TCM with good efficacy and few adverse reactions are gradually highlighted. The Ru-Pi-Xiao comes from the prescription of old Chinese medicine practitioners and is mainly available in tablets, capsules and granules, which are composed of fifteen traditional Chinese medicines. The formula has the function of promoting blood circulation, removing blood stasis, softening and resolving hard mass and clearing heat toxins. Clinically, it is mainly used for treating Ru-Pi and Ru-Yong caused by the mutual knotting of phlegm and heat. The main symptoms are breast nodules, varying in number, size and shape, soft texture or postpartum breast lumps, redness, heat and pain[9]. However, due to the multi-component, multi-target, multi-pathway and synergistic effects of TCM, the mechanism of Ru-Pi-Xiao treatment of MGH is still unclear, which limits its clinical application[10].

In the process of exploring the mechanism of action of TCM in the human body, network pharmacology has been introduced, which can comprehensively study the efficacy and mechanism of action of TCM by tapping into the massive resources of databases. So, that people can more intuitively and conveniently understand the process of action of medicines and provide the basis for optimizing the use of medicines[11,12]. At present, there is no mechanism study on Ru-Pi-Xiao for the treatment of MGH at domestic and international. This study, investigated the active ingredients and molecular mechanism of Ru-Pi-Xiao with the help of network pharmacology and molecular docking to clarify its medicinal value and to provide a good basis for the use of Ru-Pi-Xiao in the treatment of MGH.

MATERIALS AND METHODS

Study area and duration: The study was conducted from May to October, 2024 at a Laboratory in the College of Pharmacy at Anhui University of Traditional Chinese Medicine, China.

Screening of active ingredients and targets of Ru-Pi-Xiao: The compounds of Antlers, Dandelion, Kombu, Mongolian Snakegourd Root, Suberect Spatholobus Stem, Notoginseng, Red Peony Root, Seaweed, Uniflower Swisscentaury Root, Costus Root, Figwort Root, Tree Peony Bark, Common Selfheal Spike, Forsythia and Safflower in Ru-Pi-Xiao were obtained from the followed databases, namely Traditional Chinese Medicine Systems Pharmacology[13-15] and Traditional Chinese Medicine Information Database. The amount of pharmaceutical ingredient that enters the circulatory system after administration is referred to as oral bioavailability (OB). Drug similarity (DL) is the similarity between a chemical and a known drug. Filters for retrieving pharmacokinetic data were set to OB $\geq$40% and DL $\geq$0.18. Based on the obtained chemical constituents, the Smiles numbers of the constituents were obtained by searching in the PubChem database. The smiles numbers were entered into the Swiss Target Prediction to predict the component targets[16-18].

Identification of predicted targets of MGH: "Atypical Ductal Breast Hyperplasia", "Atypical Lobular Breast Hyperplasia", "Usual Ductal Hyperplasia of the Breast" and "Hyperplasia of Mammary Glands" were used as keywords in the Gene Cards Database[19], DisGeNET[20-23] Database and National Center for Biotechnology Information Databases to obtain relevant targets of MGH[11]. These databases' targets were merged, then duplicate targets were removed and the remaining targets were the MGH targets collected.

Targets intersection between active compounds and disease: The screened Ru-Pi-Xiao active ingredient targets and MGH disease targets were inputted into the online tool Venny 2.1 and the intersecting genes were obtained by constructing a Venn diagram through mapping. The nodes that play a role can be inferred from the obtained intersecting genes, which can be used as the predicted targets of drug action in the disease for the enrichment analysis of the following pathways.

Protein-protein interaction (PPI) network construction: Input the drug-disease common targets into the STRING database to obtain information on the protein interaction network. The screening condition of the organism was set to "Homo sapiens" and the minimum required interaction score was set to "Highest confidence >0.900". Input PPI information into Cytoscape 3.7.2 for visualization and construct a PPI network. Cytoscape can calculate the parameters of each node in the network diagram, using the Network Analysis plug-in to analyze the obtained PPI network and the core targets were obtained by filtering with a threshold value greater than "degree", "closeness" and "betweenness".

Compound drug-active ingredient-target network diagrams: After mapping the drug and its active ingredients with the drug-disease common targets in Excel and importing it into Cytoscape 3.7.2, the Nodes in the network graph represent the drug, active ingredient and target protein and the Edges represent the interactions between the drug and the ingredient or between the ingredient and the target protein, to construct the network graph of the compound drug-active ingredient-target.

GO enrichment analysis and KEGG pathway enrichment analysis: To study the biological function of potential targets in MGH, Metascape, database was used to collect gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) data. The biological species was set as "Homo sapiens". The common targets of Ru-Pi-Xiao active ingredients and MGH were analyzed by GO function and KEGG pathway enrichment. The results of the GO classification enrichment analysis included three parts, molecular function (MF), biological process (BP) and cellular component (CC). The top 20 entries were all selected as the analyzed objects in this study and the enrichment bubble diagrams and bar charts were plotted using the web platform, respectively.

Molecular docking: Molecular docking is a common method for drug discovery and a theoretical simulation method for studying protein-ligand interactions and recognition, which can be used to analyze the interactions between small distributors and biomolecule receptors and predict their binding modes and affinities, which in turn is important for the molecular mechanisms of pharmacological activity, structural prediction of protein-ligand complexes and the screening of targeted drugs[24]. Therefore, a molecular docking approach was used to verify whether the core components identified by network pharmacology bind to the core protein targets.

Based on the results of the network pharmacology screening, the core components with potential targets of action ranked in the top 5 of the PPI network analysis in terms of degree value were selected. The 3D structure of the target protein was retrieved from the Protein Data Bank and its PDB format file was downloaded[25]. The protein was subjected to the removal of water and replacement with hydrogen, then was designated as a receptor and the structure was saved as a PDBQT Protein Receptor file in AutoDock 4.2[26]. The structure of the active ingredient of the small molecule of the drug was downloaded in the TCMSP database and the same was done in AutoDock 4.2 by removing water, adding H and designating the small molecule as ligand and exporting the PDBQT ligand file. Molecular docking was performed using AutoDock 4.2 software and visualized using PyMOL software.

RESULTS

Ru-Pi-Xiao active ingredient and target screening: The chemical constituents of the 15 herbs were searched through the database. The screening conditions were OB $\geq$40% and DL $\geq$0.18. 1 active ingredient of Antlers, 6 active ingredients of Dandelion, 6 active ingredients of Kombu, 1 active ingredient of Mongolian Snakegourd Root, 16 active ingredients of Suberect Spatholobus Stem, 4 active ingredients of Sanchi, 17 active ingredients of Paeoniae Radix Rubra, 4 active ingredients of Seaweed, 2 active ingredients of Uniflower Swisscentaury Root, 3 active ingredients of Aucklandia Lappa, 3 active ingredients of Figwort Root, 9 active ingredients of Tree Peony Bark, 8 active ingredients of Common Selfheal Spike, 17 active ingredients of Weeping Forsythia Capsule and 13 active ingredients of Safflower were obtained. A total of 110 Ru-Pi-Xiao active ingredients were obtained after merging and de-weighting (Table 1). The target information of the 110 active ingredients was searched through the Swiss Target Prediction database and 825 targets were obtained after counting and deleting duplicates.

MGH disease-related target search results: The Gene Card database retrieved 624 related genes. The DisGeNET database retrieved 93 related genes and the NCBI database retrieved 32 related genes. The integration and de-emphasis of these three databases yielded 684 MGH-related genes.

Table 1: Potential active ingredients of "Ru-Pi-Xiao"

Drug	Active ingredients	OB (oral bioavailability)	DL (oral bioavailability)
Kombu	Saringosterol	43.47	0.62
Kombu	Eckol	87.06	0.63
Kombu	1553-41-9	45.65	0.2
Kombu	24-Methylenecholesterol	43.53	0.76
Kombu	Arachidonic acid	45.57	0.2
Kombu	Fucosterol	43.77	0.75
Mongolian snakegourd root	Spinasterol	42.97	0.75
Suberect spatholobus stem	Formononetin	69.67	0.21
Suberect spatholobus stem	Calycosin	47.75	0.24
Suberect spatholobus stem	Stigmasterol	43.82	0.75
Suberect spatholobus stem	3,7-dihydroxy-6-methoxy-dihydro flavonol	43.79	0.25
Suberect spatholobus stem	8-o-Methylreyusi	70.31	0.27
Suberect spatholobus stem	3-Hydroxystigmast-5-en-7-one	40.92	0.78
Suberect spatholobus stem	Aloe-emodin	83.37	0.24
Suberect spatholobus stem	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl) ethyl] acrylamide	118.34	0.26
Suberect spatholobus stem	(+)-catechin	54.82	0.24
Suberect spatholobus stem	Licochalcone A	40.78	0.28
Suberect spatholobus stem	Vestitol	74.65	0.2
Suberect spatholobus stem	Consume close grain	68.12	0.27
Suberect spatholobus stem	Cajinin	68.79	0.27
Suberect spatholobus stem	Medicagol	57.48	0.59
Suberect spatholobus stem	Lupinidine	61.89	0.2
Suberect spatholobus stem	Psi-Baptigenin	70.11	0.31
Notoginseng	Mandenol	41.99	0.19
Notoginseng	Diop	43.59	0.39
Notoginseng	Stigmasterol	43.82	0.75
Notoginseng	Quercetin	46.43	0.27
Red peony root	Ellagic acid	43.06	0.43
Red peony root	Paeoniflorigenone	87.59	0.36
Red peony root	Lactiflorin	49.12	0.79
Red peony root	Paeoniflorin	53.87	0.78
Red peony root	Paeoniflorin_qt	68.17	0.39
Red peony root	Baicalin	40.12	0.75
Red peony root	Spinasterol	42.97	0.75
Red peony root	Stigmasterol	43.82	0.75
Red peony root	(+)-catechin	54.82	0.24
Red peony root	(2R,3R)-4-methoxyl-distylin	59.98	0.29
Red peony root	1-o-beta-d-glucopyranosyl paeonisuffrone_qt	65.08	0.35
Red peony root	Albiflorin_qt	48.70	0.32
Red peony root	4-ethyl-paeoniflorin_qt	56.86	0.44
Red peony root	4-o-methyl-paeoniflorin_qt	56.70	0.42
Red peony root	Paeoniflorigenone	65.33	0.36
Red peony root	9-ethyl-neo-paeoniflorin A_qt	64.41	0.29
Red peony root	Evofolin B	64.73	0.22
Seaweed	N-[(1S)-1-(benzyl)-2-[[(1S)-1-(benzyl)-2-hydroxy -ethyl] amino]-2-keto-ethyl] benzamide	45.75	0.43
Seaweed	Diglycol dibenzoate	59.21	0.27
Seaweed	Isofucosterol	43.77	0.75
Seaweed	Quercetin	46.43	0.27
Uniflower swisscentaury root	Liquiritin	65.69	0.73
Uniflower swisscentaury root	Diosbulbin B	43.01	0.7
Costus root	Cynaropicrin	67.50	0.38
Costus root	Mairin	55.37	0.77
Costus root	Stigmasterol	43.82	0.75
Figwort root	Paeoniflorin_qt	68.17	0.39
Figwort root	14-deoxy-12(R)-sulfoandrographolide	62.56	0.42
Figwort root	Harpagoside_qt	122.86	0.31
Tree peony bark	Paeoniflorin_qt	68.17	0.39
Tree peony bark	Mairin	55.37	0.77

Table 1: Continue

Drug	Active ingredients	OB (oral bioavailability)	DL (oral bioavailability)
Tree peony bark	Kaempferol	41.88	0.24
Tree peony bark	(+)-catechin	54.82	0.24
Tree peony bark	4-O-methylpaeoniflorin_qt	67.23	0.42
Tree peony bark	5-[[5-(4-methoxyphenyl)-2-furyl] methylene barbituric acid	43.44	0.3
Tree peony bark	Mudanpioside-h_qt 2	42.35	0.37
Tree peony bark	Paeonidanin_qt	65.31	0.34
Tree peony bark	Quercetin	46.43	0.27
Common selfheal spike	Kaempferol	41.88	0.24
Common selfheal spike	Spinasterol	42.97	0.75
Common selfheal spike	Stigmasterol	43.82	0.75
Common selfheal spike	Delphinidin	40.63	0.27
Common selfheal spike	Vulgaxanthin-I	56.13	0.25
Common selfheal spike	Poriferasterol monoglucoside_qt	43.82	0.75
Common selfheal spike	Morin	46.22	0.27
Common selfheal spike	Quercetin	46.43	0.27
Forsythia	20(S)-dammar-24-ene-3β,20-diol-3-acetate	40.22	0.82
Forsythia	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl) -7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.38
Forsythia	(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl) methyl] oxolan-2-one	52.30	0.48
Forsythia	(+)-pinoresinol monomethyl ether	53.08	0.56
Forsythia	ACon1_001697	85.11	0.56
Forsythia	(+)-c monomethyl ether-4-D-beta-glucoside_qt	61.20	0.56
Forsythia	Mairin	55.37	0.77
Forsythia	Forsythinol	81.24	0.56
Forsythia	(-)-Phillygenin	95.03	0.56
Forsythia	β-amyrin acetate	42.06	0.74
Forsythia	Hyperforin	44.03	0.59
Forsythia	Adhyperforin	44.03	0.61
Forsythia	Lactucasterol	40.99	0.84
Forsythia	Onjixanthone I	79.15	0.29
Forsythia	Kaempferol	41.88	0.24
Forsythia	Bicuculline	69.66	0.88
Forsythia	Quercetin	46.43	0.27
Safflower	Flavoxanthin	60.41	0.55
Safflower	4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2, 5-dienylidene) but-2-enylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one	48.46	0.36
Safflower	Lignan	43.31	0.65
Safflower	Phytofluene	43.18	0.5
Safflower	Pyrethrin II	48.35	0.35
Safflower	6-Hydroxykaempferol	62.13	0.27
Safflower	qt_carthamone	51.02	0.2
Safflower	quercetagetin	45.00	0.3
Safflower	7,8-dimethyl-1H-pyrimido[5,6-g] Quinoxaline-2,4-dione	45.75	0.18
Safflower	Baicalin	40.12	0.75
Safflower	Kaempferol	41.88	0.24
Safflower	Stigmasterol	43.82	0.75
Safflower	Quercetin	46.43	0.27
Dandelion	Flavoxanthin	60.41	0.55
Dandelion	Stigmasterol	43.82	0.75
Dandelion	Vitamin D	45.65	0.47
Dandelion	3,7,11,15-Tetramethylhexadec-2-En-1-Ol	49.55	0.47
Dandelion	Hesperetin	70.31	0.27
Dandelion	Quercetin	46.43	0.27
Antlers	Estrone	53.56	0.31

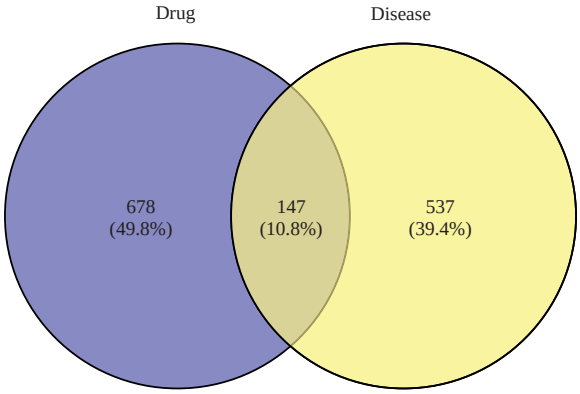

Fig. 1: Drug target-disease target Venny diagram

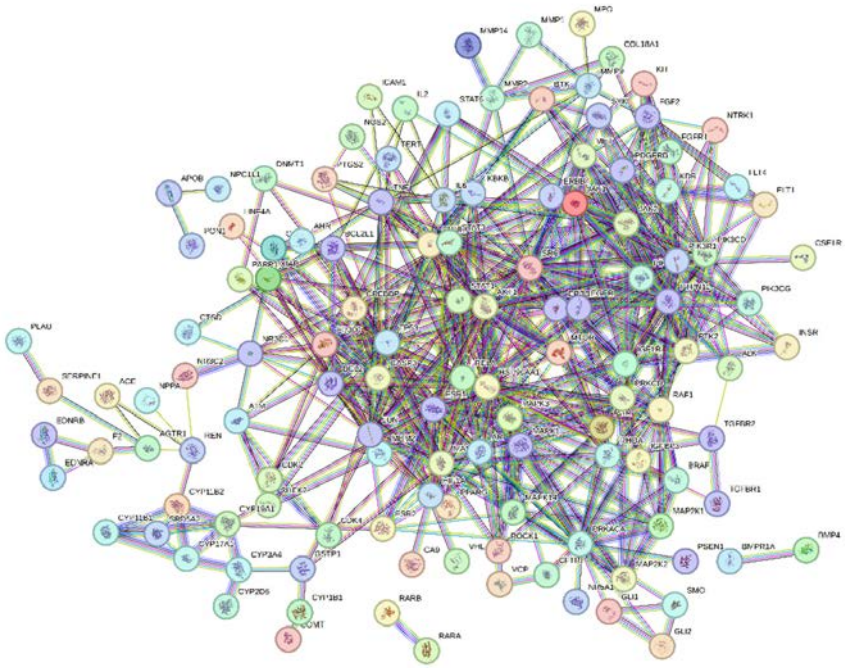

Fig. 2: MGH (mammary gland hyperplasia) and Ru-Pi-Xiao shared target PPI network done by STRING platform

Intersection of Ru-Pi-Xiao active ingredient targets and breast hyperplasia disease targets: The screened drug targets and disease targets were entered into the online tool Venny 2.1 and 147 shared targets were obtained, as shown in Fig. 1. The following pathway enrichment analysis was performed as a predictive target for drug action on disease.

PPI network construction: The drug-disease shared targets were entered into the STRING database for the construction of the PPI network and the organism species was set as "Homo sapiens" and the minimum interaction threshold was "Highest confidence >0.900" to get the PPI network. There were 147 nodes and 563 edges in the network and the average node degree value was 7.66. Figure 2 shows the PPI network graph exported from the STRING website, in which the circular nodes represent the potential targets and the edges represent the interconnections between the targets. The obtained PPI networks were analyzed using the Network Analysis plug-in of Cytoscape 3.7.2 and the core targets were obtained by filtering with thresholds greater than "degree", "closeness" and "betweenness" to obtain the core targets (Table 2) and a total of 23 core targets were filtered out. These 23 core target points were drawn using Cytoscape 3.7.2, resulting in 23 nodes and 124 edges (Fig. 3).

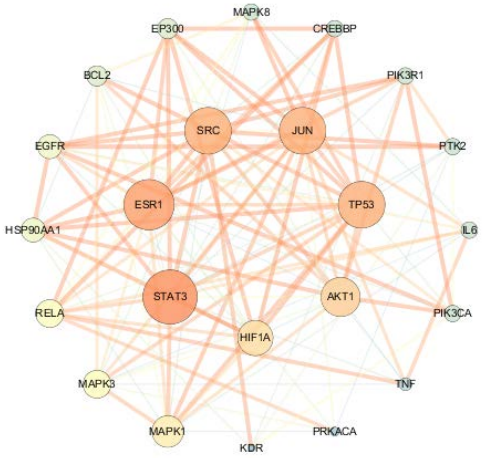

Fig. 3: Core target map made by Cytoscape 3.7.2 platform

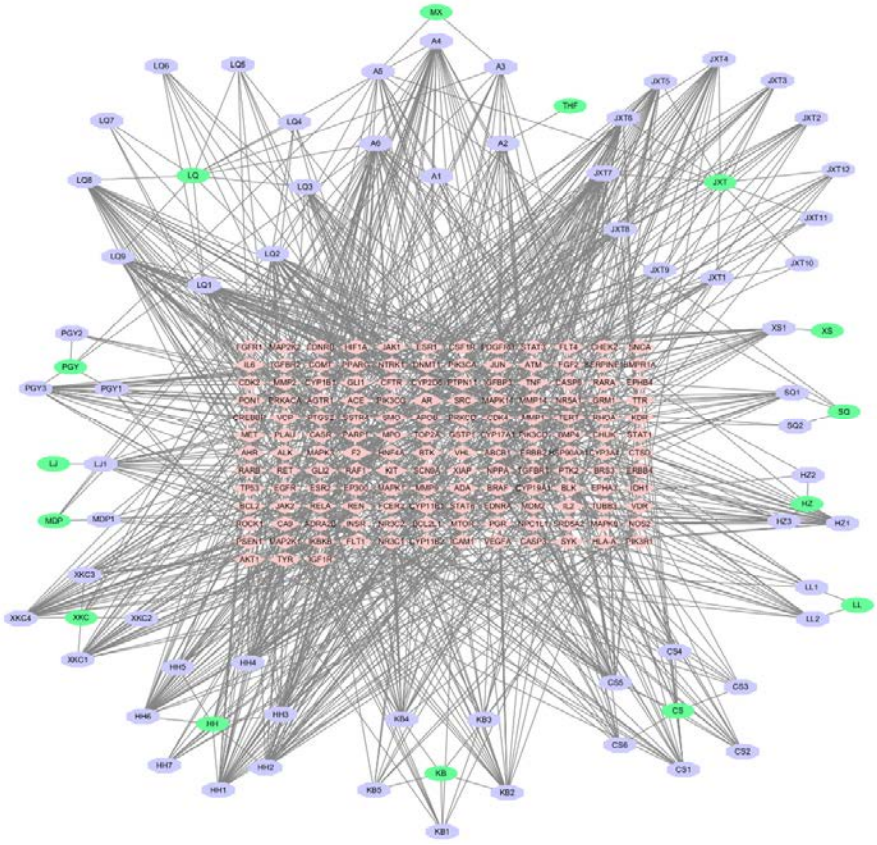

Fig. 4: Compound drug-active ingredient-targets network diagram

Compound drug-active ingredient-target network: The 15 herbal medicines, their active ingredients and drug-disease common targets action relationships were imported into Cytoscape 3.7.2 to obtain the compound drug-active ingredient-target network graph. The network contained 224 nodes and 972 edges. The results were shown in Fig. 4, in which the pink rhombus nodes represent the targets, the blue hexagonal nodes represent the active ingredients and the oval green nodes represent the drugs. The network graph illustrates that Ru-Pi-Xiao exerts its therapeutic effects on MGH through multi-components, multi-targets and multi-links.

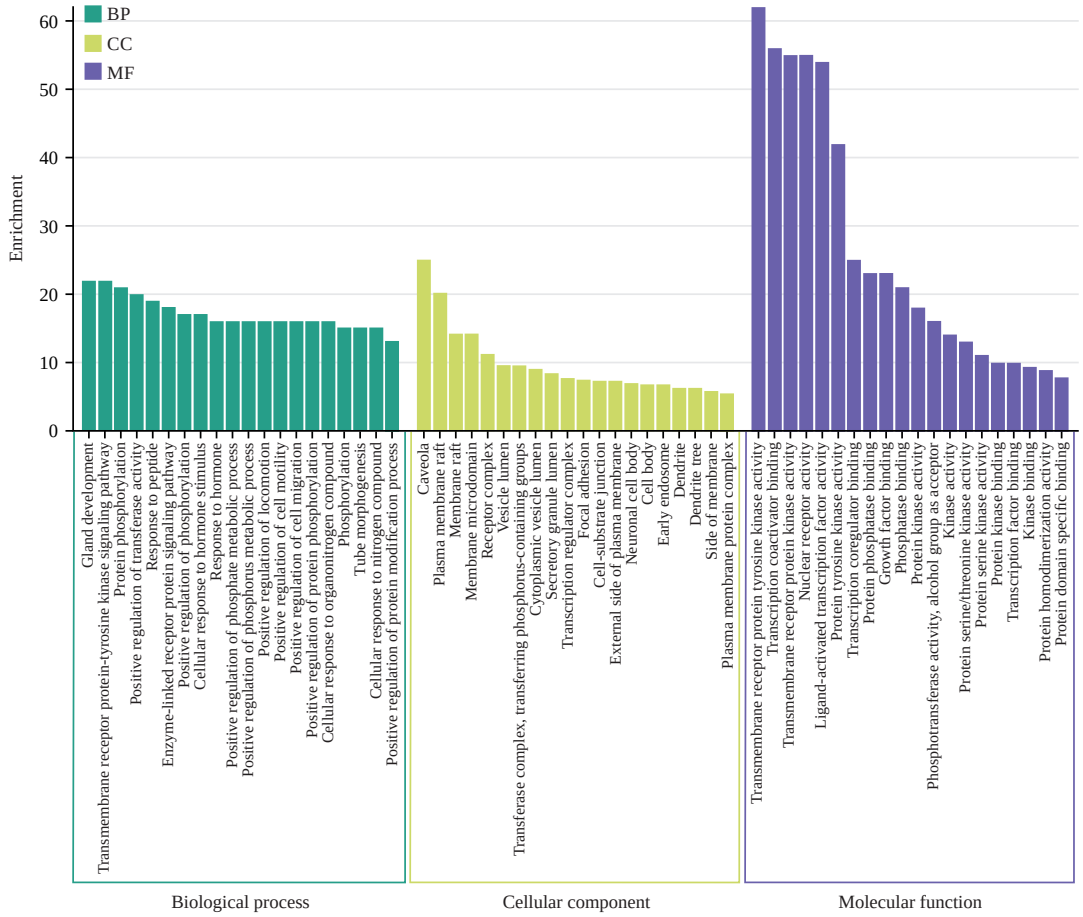

Fig. 5: Histogram of GO enrichment analysis

Table 2: Total number of core targets obtained after filtering

Number	Target	Betweenness	Closeness	Degree
1	ESR1	1632.320376	0.004065041	28
2	HSP90AA1	1352.748659	0.003802281	27
3	AKT1	847.3316014	0.003846154	30
4	MAPK1	343.7405873	0.003703704	23
5	MAPK3	316.5573786	0.003649635	22
6	EP300	375.014886	0.003584229	18
7	PIK3CA	546.0222383	0.003690037	30
8	STAT3	1576.840645	0.004132231	35
9	TP53	1655.38545	0.004032258	36
10	JUN	836.0097301	0.003846154	21
11	SRC	1157.934478	0.003921569	36
12	BCL2	265.5983938	0.003412969	16
13	TNF	350.5360838	0.003378378	16
14	PIK3R1	710.5196082	0.003610108	30
15	HIF1A	491.0853742	0.003508772	16
16	CREBBP	367.7013216	0.003558719	17
17	PRKACA	1303.624192	0.003378378	21
18	MAPK8	479.3164838	0.003521127	18
19	RELA	250.1613158	0.003533569	16
20	KDR	248.7375181	0.003267974	14
21	EGFR	273.7567007	0.003636364	23
22	IL6	337.4580461	0.003333333	16
23	PTK2	406.8892437	0.003508772	20

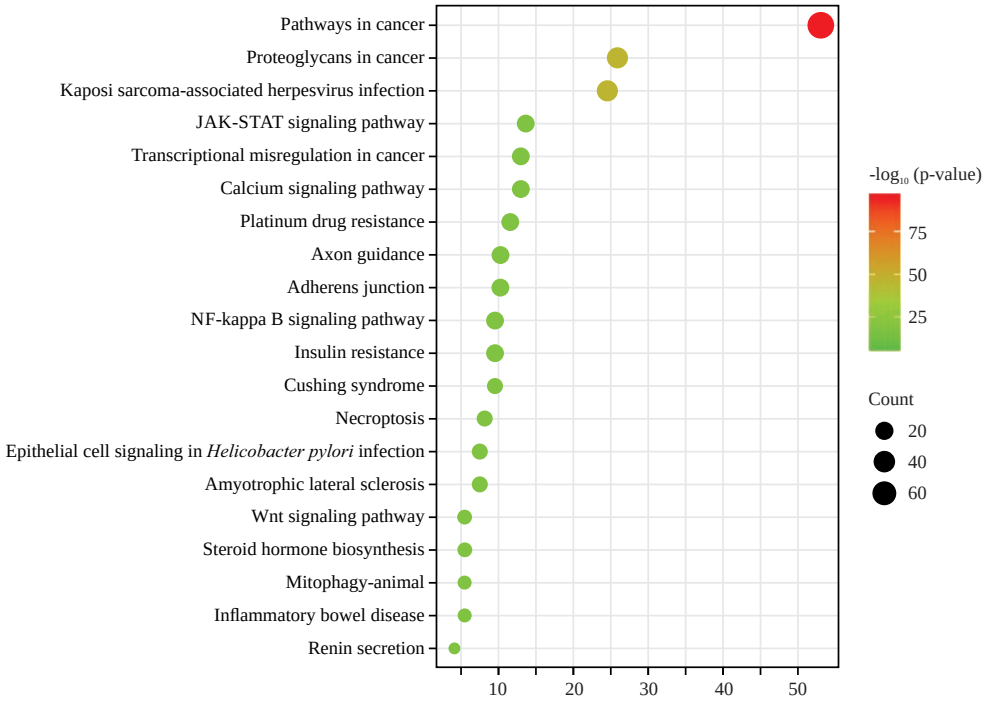

Fig. 6: Bubble diagram for KEGG enrichment analysis

Table 3: Compound and core target binding energies (kcal/mol)

Compound	Quercetin	Kaempferol	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7 -methoxy-2,3-dimethylol-tetralin-6-ol	6-Hydroxykaempferol	Lignan
TP53	-6.07	-6.00	-4.82	-5.65	-5.09
SRC	-5.95	-6.44	-6.22	-6.56	-5.85
STAT3	-4.46	-5.14	-4.66	-4.38	-3.67
AKT1	-6.98	-5.90	-4.76	-6.26	-4.61
PIK3CA	-6.63	-6.58	-6.03	-6.78	-4.82

Results of GO enrichment analysis and KEGG pathway enrichment analysis: The drug-disease shared targets were enriched for the biological process (BP), molecular function (MF) and cell component (CC) of GO. It was found that the biological process of Ru-Pi-Xiao for MGH treatment included "Response to peptide", "Transmembrane receptor protein tyrosine kinase signaling pathway", "Protein phosphorylation", "Gland development", "Positive regulation of transferase activity", etc. The molecular function was "Transcription coactivator binding", "Transmembrane receptor protein kinase activity", "Nuclear receptor activity", "Ligand-activated transcription kinase activity", "Transmembrane receptor protein tyrosine kinase activity", etc. The cellular components were "Receptor complexes", "Plasma membrane raft", "Membrane raft", "Caveola", "Membrane microdomain" and so on. Take the top 20 pieces of information and use the web platform to draw a bar graph (Fig. 5). The KEGG pathway analysis yielded 251 pathways and the top 20 pathways were obtained by screening for bubble diagrams (Fig. 6). "Pathways in cancer", "Proteoglycans in cancer", "JAK-STAT signaling pathway", "Transcriptional misregulation in cancer" and "Calcium signaling pathway" were included, which reveals that Ru-Pi-Xiao was used to treated MGH through different targets acting on different pathways.

Molecular docking results: Based on the network pharmacology results, the top five compounds ranked by Cytoscape in terms of Degree were screened out as Quercetin, Kaempferol, (2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol,6-Hydroxykaempferol, Lignan, respectively, which were performed molecular docking with the top five Degree ranked proteins in the PPI network (TP53, SRC, STAT3, AKT1, PIK3CA) to validate their interaction activities. A good binding activity between the ligand and receptor is indicated if the binding energy is less than -5 kcal/mol, furthermore, a stronger binding activity is indicated if the binding energy is less than -7 kcal/mol. Table 3 shows the docking results between compounds and proteins. Figure 7(a-e) depicts the image of the optimal docking of receptors and ligands after visualization.

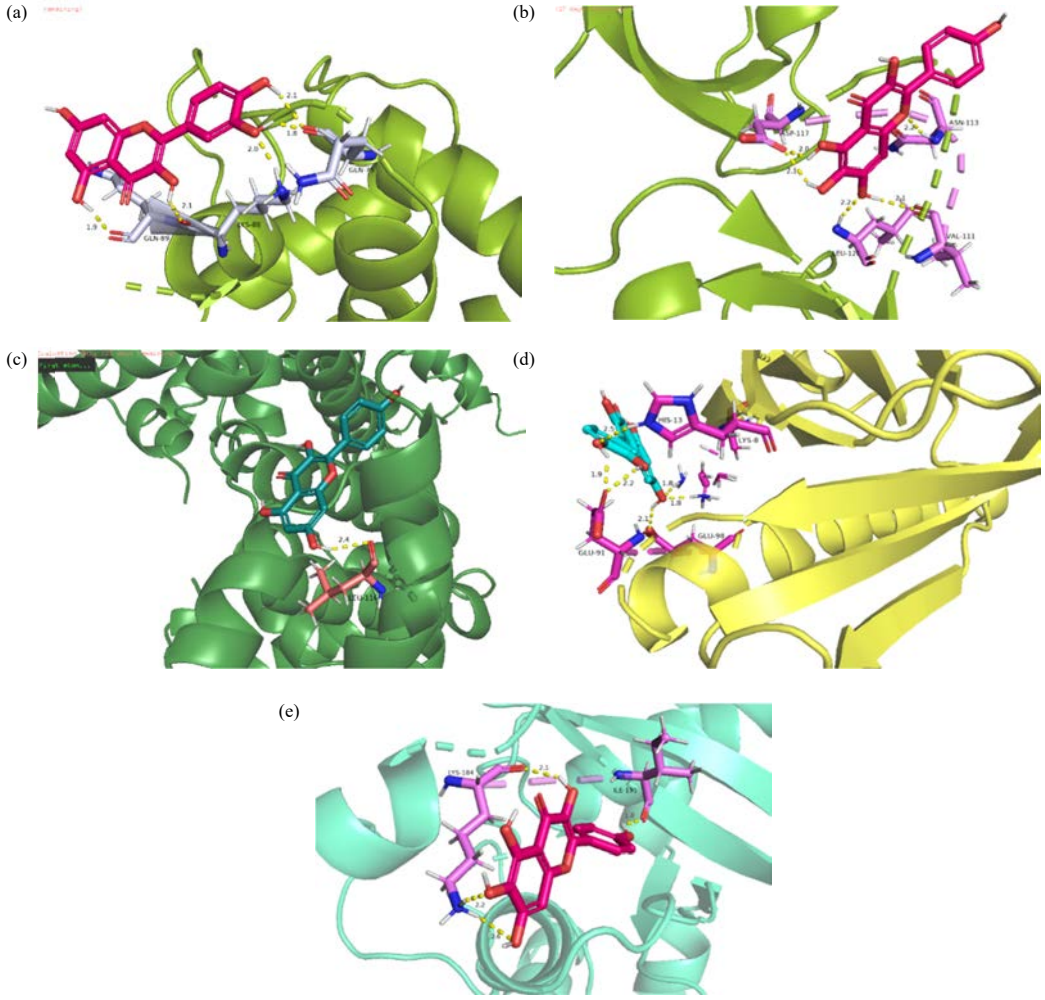

Fig. 7(a-e): Molecular docking pattern of the core components, (a) Quercetin-TP53, (b) 6-Hydroxykaempferol-SRC, (c) Kaempferol-STAT3, (d) Quercetin-AKT1 and (e) 6-Hydroxykaempferol-PIK3CA

Table 3 presents the binding affinities of different compounds to various target proteins. Among the compounds, Quercetin showed the strongest binding to AKT1 (-6.98) and TP53 (-6.07). Kaempferol demonstrated strong binding to SRC (-6.44) and STAT3 (-5.14). The third compound, (2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol, had moderate binding across all proteins, with the lowest affinity for TP53 (-4.82). The 6-Hydroxykaempferol showed consistent but relatively weaker binding, particularly to PIK3CA (-6.78) and SRC (-6.56). Lignan had the least binding overall, with its highest affinity for SRC (-5.85) and the lowest for STAT3 (-3.67).

DISCUSSION

Modern medicine considers MGH to be the most common disease in middle-aged women. Due to the accelerated pace of modern life and the increase in work and competitive pressure, the incidence of MGH has been increasing and it may develop into breast cancer in severe cases[6]. Western drugs treat MGH mainly with tamoxifen, which was used to regulate patients' endocrine secretion and was prone to various adverse reactions such as menstrual disorders and irritability[27]. According to TCM theory, MGH was related to the stagnation of liver Qi and disharmony between Chong and Ren[28]. The main treatment of MGH was to regulate Chong and Ren, activate Qi and blood circulation and soften and dissipate knots. The efficacy of Ru-Pi-Xiao in the treatment of MGH has been confirmed[27,29], but the mechanism of its action was not yet clear. So, this paper constructs a "compound drug-component-target" network based on the network pharmacology to analyze the effect of Ru-Pi-Xiao in treating MGH. Meanwhile, this paper explores the main pharmacodynamic components of Ru-Pi-Xiao, which can be

used to improve the efficacy and reduce the side effects of the preparation through the establishment of better-quality standards, the conduction of stricter quality control, the clarification of *in vivo* pharmacokinetic process and also be used to provide a basis for the development of new dosage forms of the drug.

The top 5 key targets obtained were selected as "TP53", "SRC", "STAT3", "AKT1" and "PIK3CA". Among human genes, the TP53 gene was a major player in cancer formation and was considered as the most important tumor suppressor gene. Somatic mutation in TP53 gene was one of the most common mutations in human cancers including breast carcinomas, ovarian cancer and lung and adrenal cortical cancers. However, it was also a target for drug intervention[30-32]. The SRC tyrosine-protein kinase was a proto-oncogene that plays a key role in cell morphology, motility, proliferation and survival[33-35]. The SRC expression levels were significantly elevated in breast tumors, while knockdown of c-SRC inhibits mammary ductal growth[36,37], which was a potential pathway for treating MGH. The STAT3 plays an important role in breast epithelial cell differentiation, proliferation and apoptosis. The STAT3 was usually activated during degeneration, prompting apoptosis and returning the mammary gland to a dormant state similar to the pre-pregnancy stage. However, in cancer, STAT3 contributes to proliferation, apoptosis and angiogenesis. Therefore, STAT3 was also significantly expressed in MGH and breast cancer tissues[38,39]. The AKT1 is a protein kinase that regulates cell survival and cell cycle in various cells, including breast cancer cells AKT1 promotes the growth of normal breast tissues[40] and related studies have shown that AKT1 mutations occur before breast cancer metamorphosis and AKT1 mutations can also be seen in MGH[41,42]. Inhibition of PIK3CA expression can slow down the growth of breast cells *in vivo*. The PIK3CA overexpression can also damage the DNA of mammary cells, increase the susceptibility of mammary cells and promote mammary cell apoptosis to inhibit MGH[43]. Quercetin, Kaempferol, (2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol, 6-Hydroxykaempferol and Lignan are the key ingredients in Ru-Pi-Xiao for the treatment of MGH. Quercetin is a natural flavonoid with various properties such as antioxidant, antiviral, anti-inflammatory and anticancer[44-46], which can reduce cell proliferation, induce apoptosis and regulate the cell cycle. It is a reliable molecule in cancer therapy[47]. Kaempferol is similar to Quercetin, which is also a flavonoid, with a large number of health-related effects, such as anti-inflammatory, anticancer, antimicrobial and antioxidant. Kaempferol was used for the treatment of MGH by mediating the cellular developmental process of the Estrogen Receptor (ER)-dependent pathway during cell development, which can effectively inhibit the growth of breast cancer cell lines at micromolar concentrations[48,49]. (2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol is a lignan widely distributed in plants, with significant antitumor and antioxidant activities[50,51]. The 6-Hydroxykaempferol is mostly found in safflower, which has anti-coagulant, antioxidant, anti-inflammatory and anti-tumor effects and can promote blood circulation to achieve the effect of activating blood circulation to eliminate blood stasis[52,53]. Lignan is a plant-based compound with a variety of biological properties such as antioxidant, anti-inflammatory, anti-estrogenic and anti-cancer, which can exert anti-cancer activity by inhibiting the cell cycle inducing apoptosis and by inducing anti-invasive, anti-metastatic and anti-proliferative effects[54].

Enrichment analysis showed that Proteoglycans in cancer, the JAK-STAT signaling pathway, Calcium signaling pathway and other pathways played a role in the treatment of MGH. Proteoglycans (PG) were mostly found on the cell surface and were often used as a biomarker or a target. Manipulation of PG on cell surface becomes a viable approach for potential anti-MGH[55,56]. Signal transducer and activator of transcription STAT was phosphorylated by Janus kinase (JAK), dimerized and then translocated through the nuclear membrane into the nucleus to regulate the expression of relevant genes. Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathway was involved in the process of cell proliferation, differentiation and apoptosis and also has an important regulatory role in immune function[57]. The STAT3, as an important regulator of cell transformation and apoptosis during mammary gland development, plays a central role in breast cancer cell proliferation, invasion, metastasis and immune escape[58]. The Ca^{2+} was a ubiquitous second messenger regulating many physiological functions and dysregulation of calcium homeostasis can be present in a wide range of pathological states. The Orai3, a molecular actor mediating Store-operated Ca^{2+} entry (SOCE), has been shown to encode SOCE in a subpopulation of breast cancer cells and express estrogen receptors in mature effector T cells[59]. The above analysis shows that Ru-Pi-Xiao treats MGH mainly through the anticancer pathway, with some immunomodulatory and hormonal regulatory processes.

CONCLUSION

According to the aforementioned research, the active ingredients in Ru-Pi-Xiao, including Quercetin, Kaempferol, (2R,3R,4S)-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol, 6-Hydroxykaempferol and Lignan may

act on the key targets of TP53, SRC, STAT3, AKT1, PIK3CA and regulate the signaling pathways including Proteoglycans in cancer, JAK-STAT signaling pathways, Calcium signaling pathways to enhance immune function, regulating hormone level, regulate apoptosis and exert therapeutic effects on MGH. This study indicates that Ru-Pi-Xiao may inhibit the progression of MGH through a multi-component, multi-target and multi-pathway mechanism.

SIGNIFICANCE STATEMENT

The incidence of breast hyperplasia is the highest in female breast diseases and this disease has a high probability of developing into breast cancer. The Ru-Pi-Xiao has the function of promoting blood circulation, removing blood stasis, softening and resolving hard mass and clearing heat toxins. However, the ingredients of Ru-Pi-Xiao are complicated and the treatment process of mammary hyperplasia is ambiguous. Therefore, this study used network pharmacology and molecular docking technology to explore the role of Rupixiao in the treatment of breast hyperplasia. Quercetin, Kaempferol and 6-Hydroxykaempferol were found to regulate Proteoglycans in cancer, JAK-STAT signaling pathway calcium signaling pathway and other pathways to play a role in anti-breast hyperplasia. This study enables researchers to have a deeper understanding of the composition and efficacy of Rupixiao, provides theoretical data for further development and production of Rupixiao and provides ideas for verifying the treatment of breast hyperplasia.

ACKNOWLEDGMENTS

The authors acknowledge the financial support form Major Programs of Natural Science Research in Colleges and Universities in Anhui Province (2023AH040108), Science Research Team Program of Anhui Colleges and University (2022AH010036) and Clinical Research Project of Non-directly Affiliated Hospital of Anhui University of Traditional Chinese Medicine (2024LCRM02).

REFERENCES

1. Ma, W., Z.N. Jin, X. Wang, F.M. Fu and W.H. Guo *et al*., 2021. Clinical practice guideline for diagnosis and treatment of hyperplasia of the mammary glands: Chinese Society of Breast Surgery (CSBrS) practice guideline 2021. Chin. Med. J., 134: 1891-1893.
2. Ma, D., G. Liu, X. Zhang, Q. Zhang, T. Gao and M. Liu, 2020. Massage treatment of hyperplasia of mammary glands: A protocol for a systematic review and meta-analysis. Medicine, Vol. 99. 10.1097/MD.0000000000023601.
3. Kulkarni, U., V. Nayak, M.M. Prabhu and R. Rao, 2020. Tamoxifen-induced vasculitis. J. Oncol. Pharm. Pract., 26: 735-737.
4. Sinha, M.K., A. Barman, S. Sahu, A.K. Jha and A.A. Asharaf, 2022. Tamoxifen in mastalgia: A meta-analysis. J. Obstet. Gynaecol. Can., 44: 1084-1094.
5. Li, P., Y. Tai, L. Zhang, S. Wang and Q. Guan *et al*., 2024. Mechanisms underlying the therapeutic effects of Xiaoyaosan in treating hyperplasia of mammary glands based on network pharmacology. Medicine, Vol. 103. 10.1097/MD.0000000000036263.
6. Li, X., P. Xin, C. Wang, Z. Wang, Q. Wang and H. Kuang, 2017. Mechanisms of traditional Chinese medicine in the treatment of mammary gland hyperplasia. Am. J. Chin. Med., 45: 443-458.
7. Liu, Y.F., T. An, H. Yu, Y.Y. Fan and X.H. Pei, 2023. Xiaozheng pill exerts an anti-mammary hyperplasia effect through Raf/ERK/ELK and HIF-1α/bFGF pathways. J. Tradit. Complementary Med., 13: 600-610.
8. Wang, Y., S. Wei, T. Gao, Y. Yang and X. Lu *et al*., 2018. Anti-inflammatory effect of a TCM formula Li-Ru-Kang in rats with hyperplasia of mammary gland and the underlying biological mechanisms. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.01318.
9. Li, D., C. Wang, D. Ruan, J. Li and N. Ji *et al*., 2020. Chinese massage, Tui Na, combined with herbs improves clinical symptoms and regulates sex hormones in patients with mammary gland hyperplasia. Medicine, Vol. 99. 10.1097/MD.0000000000020300.
10. Zhang, B., X. Wang and S. Li, 2013. An integrative platform of TCM network pharmacology and its application on a herbal formula, *Qing-Luo-Yin*. Evidence-Based Complementary Altern. Med., Vol. 2013. 10.1155/2013/456747.
11. Nogales, C., Z.M. Mamdouh, M. List, C. Kiel, A.I. Casas and H.H.H.W. Schmidt, 2022. Network pharmacology: Curing causal mechanisms instead of treating symptoms. Trends Pharmacol. Sci., 43: 136-150.
12. Li, X., Z. Liu, J. Liao, Q. Chen, X. Lu and X. Fan, 2023. Network pharmacology approaches for research of Traditional Chinese Medicines. Chin. J. Nat. Med., 21: 323-332.
13. Ru, J., P. Li, J. Wang, W. Zhou and B. Li *et al*., 2014. TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. J. Cheminf., Vol. 6. 10.1186/1758-2946-6-13.
14. Zeng, L. and K. Yang, 2017. Exploring the pharmacological mechanism of Yanghe Decoction on HER2-positive breast cancer by a network pharmacology approach. J. Ethnopharmacol., 199: 68-85.

15. Zhao, L., H. Zhang, N. Li, J. Chen, H. Xu, Y. Wang and Q. Liang, 2023. Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. J. Ethnopharmacol., Vol. 309. 10.1016/j.jep.2023.116306.
16. Xue, R., Z. Fang, M. Zhang, Z. Yi, C. Wen and T. Shi, 2013. TCMID: Traditional Chinese medicine integrative database for herb molecular mechanism analysis. Nucleic Acids Res., 41: D1089-D1095.
17. Ji, Z.L., H. Zhou, J.F. Wang, L.Y. Han, C.J. Zheng and Y.Z. Chen, 2006. Traditional Chinese medicine information database. J. Ethnopharmacol., Vol. 103. 10.1016/j.jep.2005.11.003.
18. Huang, L., D. Xie, Y. Yu, H. Liu, Y. Shi, T. Shi and C. Wen, 2018. TCMID 2.0: A comprehensive resource for TCM. Nucleic Acids Res., 46: D1117-D1120.
19. Safran, M., I. Dalah, J. Alexander, N. Rosen and T.I. Stein *et al*., 2010. GeneCards version 3: The human gene integrator. Database, Vol. 2010. 10.1093/database/baq020.
20. Piñero, J., J. Saüch, F. Sanz and L.I. Furlong, 2021. The DisGeNET cytoscape app: Exploring and visualizing disease genomics data. Comput. Struct. Biotechnol. J., 19: 2960-2967.
21. Piñero, J., J.M. Ramírez-Anguita, J. Saüch-Pitarch, F. Ronzano, E. Centeno, F. Sanz and L.I. Furlong, 2020. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res., 48: D845-D855.
22. Piñero, J., À. Bravo, N. Queralt-Rosinach, A. Gutiérrez-Sacristán and J. Deu-Pons *et al*., 2017. DisGeNET: A comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res., 45: D833-D839.
23. Piñero, J., N. Queralt-Rosinach, À. Bravo, J. Deu-Pons and A. Bauer-Mehren *et al*., 2015. DisGeNET: A discovery platform for the dynamical exploration of human diseases and their genes. Database, Vol. 2015. 10.1093/database/bav028.
24. Chen, G., A.J. Seukep and M. Guo, 2020. Recent advances in molecular docking for the research and discovery of potential marine drugs. Mar. Drugs, Vol. 18. 10.3390/md18110545.
25. Burley, S.K., C. Bhikadiya, C. Bi, S. Bittrich and L. Chen *et al*., 2021. 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., 49: D437-D451.
26. Eberhardt, J., D. Santos-Martins, A.F. Tillack and S. Forli, 2021. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. J. Chem. Inf. Model., 61: 3891-3898.
27. Li, H.T., H.H. Liu, Y.X. Yang, T. Wang and X.L. Zhou *et al*., 2018. Therapeutic effects of a traditional Chinese medicine formula plus tamoxifen vs. tamoxifen for the treatment of mammary gland hyperplasia: A meta-analysis of randomized trials. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.00045.
28. Ma, M., L. Zhang and X. Wang, 2021. Effect of auricular point pressing therapy on hyperplasia of mammary glands: A protocol for systematic review and meta-analysis. Medicine, Vol. 100. 10.1097/MD.0000000000024875.
29. Wang, L., D. Zhao, L. Di, D. Cheng, X. Zhou, X. Yang and Y. Liu, 2011. The anti-hyperplasia of mammary gland effect of *Thladiantha dubia* root ethanol extract in rats reduced by estrogen and progestogen. J. Ethnopharmacol., 134: 136-140.
30. Olivier, M., M. Hollstein and P. Hainaut, 2010. TP53 mutations in human cancers: Origins, consequences, and clinical use. Cold Spring Harbor Perspect. Biol., Vol. 2. 10.1101/cshperspect.a001008.
31. Voskarides, K. and N. Giannopoulou, 2023. The role of *TP53* in adaptation and evolution. Cells, Vol. 12. 10.3390/cells12030512.
32. Wang, Z., A. Strasser and G.L. Kelly, 2022. Should mutant TP53 be targeted for cancer therapy? Cell Death Differ., 29: 911-920.
33. de Kock, L. and K. Freson, 2020. The (patho)biology of SRC kinase in platelets and megakaryocytes. Medicina, Vol. 56. 10.3390/medicina56120633.
34. Raji, L., A. Tetteh and A.R.M. Ruhul Amin, 2024. Role of c-Src in carcinogenesis and drug resistance. Cancers, Vol. 16. 10.3390/cancers16010032.
35. Bagnato, G., M. Leopizzi, E. Urciuoli and B. Peruzzi, 2020. Nuclear functions of the tyrosine kinase Src. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21082675.
36. Lydon, J.P. and B.W. O'Malley, 2011. Minireview: Steroid receptor coactivator-3: A multifarious coregulator in mammary gland metastasis. Endocrinology, 152: 19-25.
37. Marcotte, R., H.W. Smith, V. Sanguin-Gendreau, R.V. McDonough and W.J. Muller, 2011. Mammary epithelial-specific disruption of c-Src impairs cell cycle progression and tumorigenesis. Proc. Natl. Acad. Sci. U.S.A., 109: 2808-2813.
38. Shi, A., J. Dong, S. Hilsenbeck, L. Bi, H. Zhang and Y. Li, 2015. The status of STAT3 and STAT5 in human breast atypical ductal hyperplasia. PLoS ONE, Vol. 10. 10.1371/journal.pone.0132214.
39. Jhan, J.R. and E.R. Andrechek, 2016. Stat3 accelerates Myc induced tumor formation while reducing growth rate in a mouse model of breast cancer. Oncotarget, 7: 65797-65807.
40. Ackler, S., S. Ahmad, C. Tobias, M.D. Johnson and R.I. Glazer, 2002. Delayed mammary gland involution in MMTV-AKT1 transgenic mice. Oncogene, 21: 198-206.
41. Ma, W., B. Shi, F. Zhao, Y. Wu and F. Jin, 2019. Systematic analysis of breast atypical hyperplasia-associated hub genes and pathways based on text mining. Eur. J. Cancer Prev., 28: 507-514.
42. Jahn, S.W., K. Kashofer, A. Thüringer, L. Abete and E. Winter *et al*., 2016. Mutation profiling of usual ductal hyperplasia of the breast reveals activating mutations predominantly at different levels of the PI3K/AKT/mTOR pathway. Am. J. Pathol., 186: 15-23.

43. Hsu, Y.H., J. Yao, L.C. Chan, T.J. Wu and J.L. Hsu *et al*., 2014. Definition of PKC-α, CDK6, and met as therapeutic targets in triple-negative breast cancer. Cancer Res., 74: 4822-4835.
44. Singh, P., Y. Arif, A. Bajguz and S. Hayat, 2021. The role of quercetin in plants. Plant Physiol. Biochem., 166: 10-19.
45. Hosseini, A., B.M. Razavi, M. Banach and H. Hosseinzadeh, 2021. Quercetin and metabolic syndrome: A review. Phytother. Res., 35: 5352-5364.
46. Alizadeh, S.R. and M.A. Ebrahimzadeh, 2022. Quercetin derivatives: Drug design, development, and biological activities, a review. Eur. J. Med. Chem., Vol. 229. 10.1016/j.ejmech.2021.114068.
47. Deepika and P.K. Maurya, 2022. Health benefits of quercetin in age-related diseases. Molecules, Vol. 27. 10.3390/molecules27082498.
48. Imran, M., B. Salehi, J. Sharifi-Rad, T.A. Gondal and F. Saeed *et al*., 2019. Kaempferol: A key emphasis to its anticancer potential. Molecules, Vol. 24. 10.3390/molecules24122277.
49. Periferakis, A., K. Periferakis, I.A. Badarau, E.M. Petran and D.C. Popa *et al*., 2022. Kaempferol: Antimicrobial properties, sources, clinical, and traditional applications. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms232315054.
50. Sampei, M., M.A. Arai and M. Ishibashi, 2018. Total syntheses of schizandriside, saracoside and (±)-isolariciresinol with antioxidant activities. J. Nat. Med., 72: 651-654.
51. Bannwart, C., H. Adlercreutz, K. Wähälä, G. Brunow and T. Hase, 1989. Detection and identification of the plant lignans lariciresinol, isolariciresinol and secoisolariciresinol in human urine. Clin. Chim. Acta, 180: 293-301.
52. Yao, D., Z. Wang, L. Miao and L. Wang, 2016. Effects of extracts and isolated compounds from safflower on some index of promoting blood circulation and regulating menstruation. J. Ethnopharmacol., 191: 264-272.
53. Wang, L.W., J.F. He, H.Y. Xu, P.F. Zhao and J. Zhao *et al*., 2022. Effects and mechanisms of 6-hydroxykaempferol 3,6-di-O-glucoside-7-O-glucuronide from safflower on endothelial injury *in vitro* and on thrombosis *in vivo*. Front. Pharmacol., Vol. 13. 10.3389/fphar.2022.974216.
54. Shimul Bhuia, M., P. Wilairatana, R. Chowdhury, A.I. Rakib and H. Kamli *et al*., 2023. Anticancer potentials of the lignan magnolin: A systematic review. Molecules, Vol. 28. 10.3390/molecules28093671.
55. Espinoza-Sánchez, N.A. and M. Götte, 2020. Role of cell surface proteoglycans in cancer immunotherapy. Semin. Cancer Biol., 62: 48-67.
56. Wei, J., M. Hu, K. Huang, S. Lin and H. Du, 2020. Roles of proteoglycans and glycosaminoglycans in cancer development and progression. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21175983.
57. Xin, P., X. Xu, C. Deng, S. Liu and Y. Wang *et al*., 2020. The role of JAK/STAT signaling pathway and its inhibitors in diseases. Int. Immunopharmacol., Vol. 80. 10.1016/j.intimp.2020.106210.
58. Xue, C., Q. Yao, X. Gu, Q. Shi and X. Yuan *et al*., 2023. Evolving cognition of the JAK-STAT signaling pathway: Autoimmune disorders and cancer. Signal Transduction Targeted Ther., Vol. 8. 10.1038/s41392-023-01468-7.
59. Motiani, R.K., J.A. Stolwijk, R.L. Newton, X. Zhang and M. Trebak, 2013. Emerging roles of Orai3 in pathophysiology. Channels, 7: 392-401.