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Molecular mechanism of QH-BJ drug pair in the treatment of systemic lupus erythematosus based on network pharmacology and molecular docking

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Abstract

To analyze the molecular mechanism of Qinghao-Biejia (QH-BJ) drug pair in the treatment of systemic lupus erythematosus (SLE) based on the method of network pharmacology and molecular docking technology. The components and related targets of QH-BJ drug pair, as well as SLE-related targets, were obtained. Intersection targets of QH-BJ drug pair and SLE were screened to construct the protein–protein interaction network, conduct gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis, and establish the component-target-pathway network. The core active components and core targets of QH-BJ drug pair for the treatment of SLE were selected, and molecular docking was carried out between the ligand components and the receptor target proteins. The core active components of QH-BJ drug pair for the treatment of SLE are luteolin, quercetin, and kaempferol; the core targets are PTGS2, HSP90AA1, RELA, MAPK1, MAPK14, AKT1, JUN, TNF, TP53. The ligand components can spontaneously bind to the receptor target proteins. Besides, QH-BJ drug pair is likely to act on PI3K/Akt signal pathway, interleukin-17 signal pathway, and TNF signal pathway in the treatment of SLE. The study indicates that QH-BJ drug pair might play a role in the treatment of SLE through multi-components, multi-targets, and multi-pathways.

Abbreviations: BJ = Biejia, GCs = glucocorticoids, GO = gene ontology, IL = interleukin, KEGG = Kyoto Encyclopedia of Genes and Genomes, NF-κB = nuclear factor-κB, PPI = protein–protein interaction, QH = Qinghao, SLE = systemic lupus erythematosus, TCM = Traditional Chinese Medicine, TNF = tumor necrosis factor.

Keywords: Biejia, molecular docking, network pharmacology, Qinghao, systemic lupus erythematosus

1. Introduction

Systemic lupus erythematosus (SLE) is a highly heterogeneous autoimmune disease characterized by the presence of diverse autoantibodies, especially anti-dsDNA antibody. The etiology of SLE is complex and still unclear. Heredity, sex hormone, environment (including virus and bacterial infection), and other factors all play important roles in the development of SLE.[1–3] The disease is common among women of childbearing age, and the female to male ratio is 10–12 to 1.[4] The prevalence rate of SLE varies dramatically among regions. It is reported that the global prevalence rate is 0 to 241 per 100,000.[5] So far, glucocorticoids (GCs) are still the main drugs for SLE treatment. However, GCs would cause metabolic disorders, infection, osteoporosis, and other side effects.[6]

Traditional Chinese medicine (TCM) is popular in China. It treats diseases by combining different herbs and usually has the advantages of multi-components, multi-targets, and multi-pathways.[7] TCM has been proven to be safe and effective in the treatment of SLE, especially in improving patients' symptoms and quality of life.[8] When combined with GCs, TCM shows a good synergistic effect by not only improving the treatment effect, but also reducing the dose of GCs and further promoting their withdrawal. Besides, it can prevent adverse reactions caused by GCs.[9,10]

Qinghao (QH)-Biejia (BJ) decoction, one of the classic prescriptions in ancient China, has been reported to be effective and safe in the treatment of SLE, by restoring body temperature, relieving joint pain, reducing erythema, and eliminating edema, etc.[8] QH and BJ are core components of this decoction and are always combined as a drug pair for the treatment of SLE. QH is the dried aerial part of *Artemisia annua* L., and BJ is the carapace of *Trionyx sinensis* Wiegmann. QH-BJ drug pair has been demonstrated to not only reduce serum autoantibodies level, urine protein, renal immune complex deposition, and the levels of inflammatory cytokines, and improve the renal

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All data generated or analyzed during this study are included in this published article [and its supplementary information files]. The datasets generated during and/or analyzed during the current study are publicly available at TCMSP database: http://tcmspw.com/tcmsp.php; Chemistry Database: http://www.organchem.csdb.cn/scdb/default.asp; GeneCards: https://www.genecards.org; OMIM: http://www.omim.org; TTD: http://bidd.nus.edu.sg/group/cjttd; DrugBank database: https://www.drugbank.ca.

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pathological changes, but also lead to a reduction in aortic atheromatous plaque and some improvement in dyslipidemia in female ApoE$^{-/-}$ mice intraperitoneally injected with pristane.[11]

The emergence of network pharmacology should be attributed to the rapid progress in bioinformatics, systems biology, and polypharmacology.[12] The network pharmacology approach has been used to study "compound-proteins/genes-disease" pathways, which are capable of describing complexities among biological systems, drugs, and diseases from a network perspective, sharing a similar holistic philosophy as TCM.[13] Therefore, this approach makes it very powerful for the analysis of drug combinations (especially TCM preparations) and is a rational approach for TCM studies.

In this study, network pharmacology was used to analyze the potential active components and targets of this drug pair in the treatment of SLE.

2. Method

This study was approved by the Experimental Animal Health Ethics Committee of Zhejiang Chinese Medical University. The flowchart of the current study is depicted in Figure 1.

2.1. Screening active compounds and related targets of QH-BJ drug pair

The active components of QH and their related targets were obtained from the Traditional Chinese Medicine Systems Pharmacology database (http://tcmspw.com/tcmsp.php).[14] The active components were screened according to oral bioavailability ≥ 30% and drug-likeness ≥ 0.18. The active components of BJ were mainly acquired from the Chemistry Database (http://www.organchem.csdb.cn/scdb/default.asp) and were further supplemented via published literature. The structural formula of each component of BJ was searched and its SDF file was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The SDF files were then imported into the SwissTargetPrediction database (http://swisstargetprediction.ch) for ADME screening and target prediction. At last, the predicted targets of QH and BJ were standardized in the Uniprot database (https://www.uniprot.org).

2.2. Acquiring related targets for SLE

SLE-related gene targets were acquired from the following databases with "systemic lupus erythematosus" as the keyword: GeneCards (https://www.genecards.org), OMIM (http://www.omim.org), TTD (http://bidd.nus.edu.sg/group/cjttd). Besides, the targets were supplemented from the DrugBank database (https://www.drugbank.ca) where we looked for the therapeutic targets for SLE.[15] Excessive targets from the GeneCards database would be screened according to the Relevance Score value, as the higher the Relevance Score value, the closer the target is associated with the disease. Gene targets from the 4 databases would be merged and the repeated ones would be deleted.

2.3. Obtaining intersection targets and constructing protein–protein interaction (PPI) network

The targets of QH-BJ drug pair, as well as SLE-related targets, were matched on ImageGP platform (http://www.ehbio.com/ImageGP), and the Wayne diagram was drawn to get the intersection part. The intersection targets were put into the STRING platform (https://string-db.org) to construct the PPI network.[16] Corresponding parameters were set as follows: the biological species was "Homosapiens," and the minimum interaction threshold was set to "highest confidence" (>0.9); the unconnected nodes in the network were hidden; the rest were set by default. The PPI network was then visualized by Cytoscape3.6.1. The core targets are selected from the PPI network according to the network topology parameters.

2.4. Conducting gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of intersection targets

Metascape platform (http://metascape.org/gp/index.html) is a powerful tool for gene functional annotation analysis.[17] The

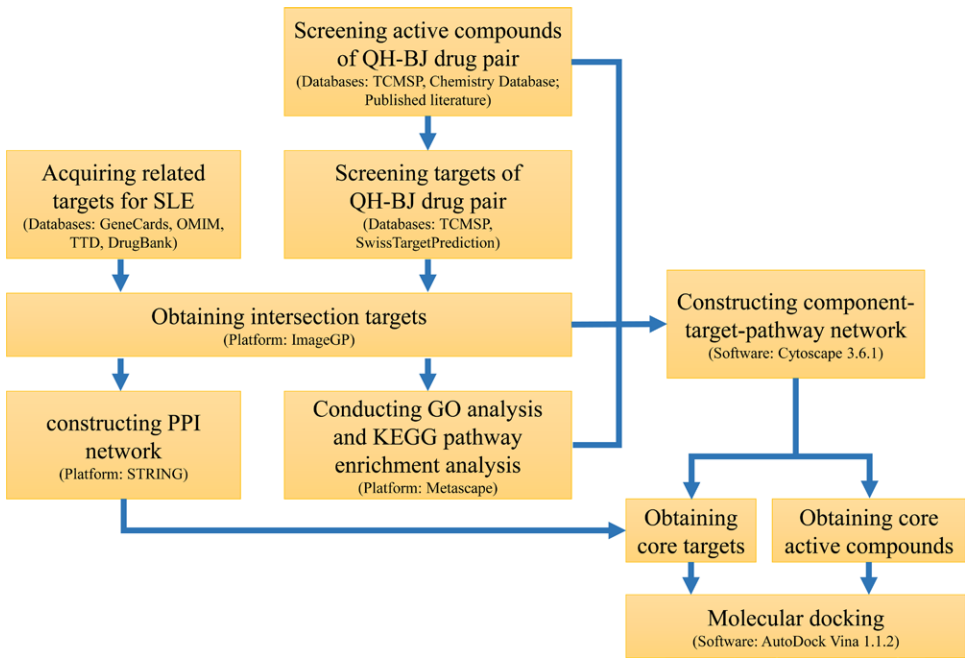

Figure 1. Flow chart of this study.

intersection targets between QH-BJ drug pair and SLE were written into Metascape. Corresponding parameters were set as follows: the "input as species" and "analysis species" items were both set to "Homo sapiens" ($P < .01$). Then GO analysis and KEGG pathway enrichment analysis were carried out, and the data results were visualized by ImageGP platform.

2.5. Constructing component-target-pathway network

The component-target-pathway network of QH-BJ drug pair for the treatment of SLE was constructed with Cytoscape 3.6.1. The network topology parameters (including "Degree," "Betweenness Centrality" and "Closeness Centrality," etc.) of active components and targets were analyzed in order to predict the core active components and core targets that play a vital role in SLE.

2.6. Molecular docking verification

Retrieve the 3D structures of the core active components and download them as SDF files from the PubChem(https://pubchem.ncbi.nlm.nih.gov/). Convert the SDF files into PDB ones with Open Babel 2.3.2. The crystal structures of the core target proteins of the PPI network and component-target-pathway network were obtained from the Protein Data Bank database (http://www.rcsb.org/pdb). These receptor proteins were dehydrated and their ligands were removed with Pymol2.3.4. Then, they were hydrogenated and their charge was balanced with AutoDock Tools. Next, the GridOption tool was used to deal with each receptor protein and pre-docked ligand molecule to determine the size and position of the binding pocket. Afterward, the receptor proteins and ligand molecules were converted into PDBQT files respectively. AutoDock Vina is an open-source program for doing molecular docking, and combines the advantages of experience-based scoring function and knowledge-based scoring function.[18] Finally, AutoDock Vina 1.1.2 was employed to dock the core active components with the core targets.

3. Results

3.1. Compounds and related targets of QH-BJ drug pair

A total of 126 chemical constituents were obtained after QH was introduced into the Traditional Chinese Medicine Systems Pharmacology, but only 19 components containing 196 related targets remained after ADME screening (Table 1). A total of 10 chemical constituents of BJ were obtained from Chemistry Database and bibliography retrieval (Table 1), and 90 related targets were acquired from SwissTargetPrediction database.

3.2. Related targets for SLE

4057 related targets for SLE were obtained from the Genecards database, among which the Relevance Score value ranged from 0.27 to 108.26 with a median of 3.48. After being screened under the condition of Relevance Score value ≥ 3.48, 2031 targets remained, among which the median Relevance Score value was 7.64. The 2031 targets were selected again in the same way, and 1016 targets were obtained and thus regarded as related targets for SLE. Besides, targets were supplemented from OMIM, TTD, and DrugBank databases, and the duplicate ones were deleted. Finally, a total of 1104 SLE-related targets were obtained.

3.3. Intersection targets between QH-BJ drug pair and SLE, and their PPI network

As shown in the Wayne diagram, a total of 118 intersection targets were acquired, including 101 targets taken from the

Table 1

Active compounds of QH-BJ drug pair.

ID	Molecule ID	Molecule name	OB (%)	DL
QH01	MOL000006	luteolin	36.16	0.25
QH02	MOL000098	quercetin	46.43	0.28
QH03	MOL000354	isorhamnetin	49.6	0.31
QH04	MOL000359	sitosterol	36.91	0.75
QH05	MOL000422	kaempferol	41.88	0.24
QH06	MOL000449	Stigmasterol	43.83	0.76
QH07	MOL002235	EUPATIN	50.8	0.41
QH08	MOL004083	Tamarixetin	32.86	0.31
QH09	MOL004112	Patuletin	53.11	0.34
QH10	MOL004609	Areapillin	48.96	0.41
QH11	MOL005229	Artemetin	49.55	0.48
QH12	MOL007274	Skrofulein	30.35	0.3
QH13	MOL007401	Cirsiliol	43.46	0.34
QH14	MOL007404	vitexin_qt	52.18	0.21
QH15	MOL007412	DMQT	42.6	0.37
QH16	MOL007415	[(2S)-2-[[(2S)-2-(benzoylamino)-3-phenylpropanoyl]amino]-3-phenylpropyl] acetate	58.02	0.52
QH17	MOL007423	6,8-di-c-glucosylapigenin_qt	59.85	0.21
QH18	MOL007424	artemisinin	49.88	0.31
QH19	MOL007426	deoxyartemisinin	54.47	0.26
BJ01	—	galactose	—	—
BJ02	—	phenylalanine	—	—
BJ03	—	alanine	—	—
BJ04	—	glutamic acid	—	—
BJ05	—	methionine	—	—
BJ06	—	proline	—	—
BJ07	—	threonine	—	—
BJ08	—	aspartic acid	—	—
BJ09	—	isoleucine	—	—
BJ10	—	histidine	—	—

BJ = Biejia, DL = drug-likeness, OB = oral bioavailability, QH = Qinghao.

intersection of QH and SLE, and 20 targets from the intersection of BJ and SLE (Fig. 2).

After the 118 intersection targets were put into the STRING platform, the PPI network was constructed with 106 nodes (Degree value ranged from 1 to 9) and 442 edges. With the help of Cytoscape3.6.1, the nodes were rearranged according to the Degree value, which was presented in the area of these nodes, that is, the higher the Degree value, the larger the node area (Fig. 3). Compared to other intersection targets, AKT1, JUN, TNF, TP53, MAPK1, RELA, and MAPK14 have higher Degree values, Betweenness Centrality values, and Closeness Centrality values, and are regarded as the core targets of the PPI network (Table 2).

3.4. GO analysis and KEGG pathway enrichment analysis of intersection targets

GO analysis and KEGG pathway enrichment analysis of intersection targets were performed on the Metascape platform, and the results were visualized with the help of ImageGP. KEGG pathway enrichment analysis indicates that the main pathways in which the intersection targets involve include PI3K/Akt signal pathway, interleukin-17 (IL-17) signal pathway, and tumor necrosis factor (TNF) signal pathway (Table 3, Fig. 4). Go analysis contains 3 dimensions, namely, molecular functions analysis, biological processes analysis, and cellular components analysis. The 3 aspects of GO analysis of the intersection targets shows that the function mainly focuses on cytokine activity, oxidoreductase activity, protein kinase binding (Fig. 5); the biological processes mainly center on blood circulation, cytokine-mediated signaling pathway, regulation of cell adhesion, response to steroid hormone (Fig. 6); in addition, the intersection targets mainly act on extracellular matrix, protein kinase complex, perinuclear region of cytoplasm, transcription factor complex and other cellular components (Fig. 7).

3.5. Predicted component-target-pathway network of QH-BJ drug pair for the treatment of SLE

With the help of Cytoscape 3.6.1, a predicted component-target-pathway network of QH-BJ drug pair for the treatment of SLE was established, with 162 nodes and 599 edges (Fig. 8).

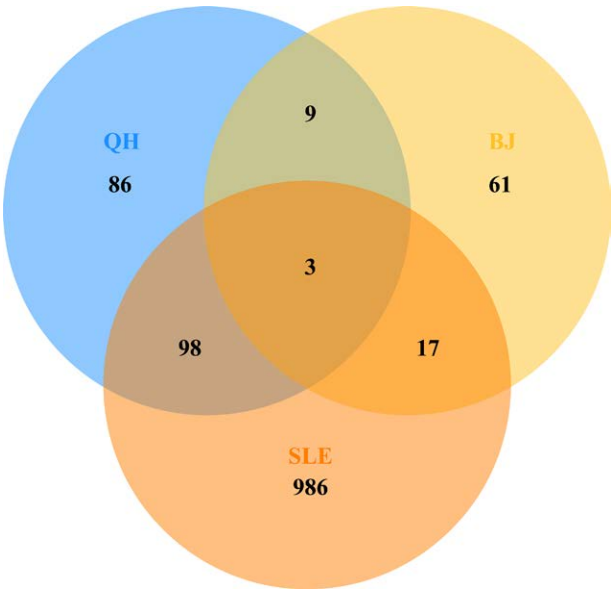

Figure 2. Intersection targets between QH-BJ drug pair and SLE. BJ = Biejia, QH = Qinghao, SLE = systemic lupus erythematosus.

NetworkAnalyzer tool within Cytoscape 3.6.1 was applied to analyze the network topology parameters in order to speculate the core components and core targets in this network. Cytoscape network analysis shows that the Degree values of luteolin, quercetin, and kaempferol, are significantly higher than those of other components, and therefore these 3 components may play the most important role in the treatment of SLE (Table 4). Compared to other intersection targets, PTGS2, HSP90AA1 and RELA have relatively higher Degree values, as well as Betweenness Centrality values and Closeness Centrality values (Table 5). These targets along with the core targets of the PPI network may be the most important targets of the QH-BJ drug pair for the treatment of SLE.

3.6. Molecular docking verification

The goal of molecular docking is to predict the bound conformations and the binding affinity.[18] Affinity is a core parameter of AutoDock Vina to assess whether the ligand can effectively bind to the receptor protein. The affinity value less than zero indicates that the ligand and receptor can bind spontaneously. The lower the affinity value, the better the binding effect. The result of molecular docking suggests that luteolin, quercetin, and kaempferol all can spontaneously bind to the core target proteins, especially PTGS2 and AKT1 (Table 6).

4. Discussion

The survival of SLE patients has increased significantly over the past decades and the 10-years survival rate is reported to exceed 90%.[1] However, when compared SLE cohorts to the general population and standardized by age and sex, the standardized mortality ratio is 2.6-fold.[19] Renal damage, cardiovascular disease, infection, and other complications are the most common causes of death observed in SLE patients.[20] Duration, dosage, and individual susceptibility of GCs and immunosuppressants can not be underestimated in the occurrence and development of complications.[8] Due to the good synergistic effect of integrated TCM and chemical medicine, this combined therapy gets more and more attention.

Drug pair, the smallest unit in TCM decoction, is a combined application of 2 herbs, which is not only simpler than the general decoction but also has the basic characteristics of decoction compatibility. It can improve the efficacy of each other or reduce the side effects of toxic herbs, and has been proved to be effective under the guidance of TCM theory. QH-BJ is a widely used drug pair in the treatment of SLE, especially for patients with Yin deficiency syndrome. It is generally agreed by contemporary Chinese medicine that Yin deficiency and endogenous heat is the core pathogenesis of SLE. Based on TCM theory, BJ can introduce QH into Yin, and QH can carry BJ out of Yang. The combination of these 2 herbs can clear heat and dispel pathogenic factors, as well as remove bone steaming and nourish Yin. It is reported that QH-BJ drug pair could significantly regulate lipid metabolism, inhibit pro-inflammatory response, and improve kidney damage of MRL/lpr mice.[21] QH and BJ are both sovereign herbs in QH-BJ decoction which is taken as a predominant prescription for SLE patients with Yin deficiency syndrome.[8] Studies have shown that the decoction can alleviate the progression of SLE by suppressing the production of Th17 cells and IL-17 in MRL/lpr mice.[22,23]

A total of 29 chemical constituents of QH-BJ drug pair were included, among which luteolin, quercetin, and kaempferol were speculated to be the core active components in the treatment of SLE. These 3 components are all flavonoids that have a wide range of beneficial effects and biological activities, particularly strong anti-oxidative and anti-inflammatory activities.[24–27] At present, no studies exist that directly verify the effects of luteolin and kaempferol on SLE. However, excessive

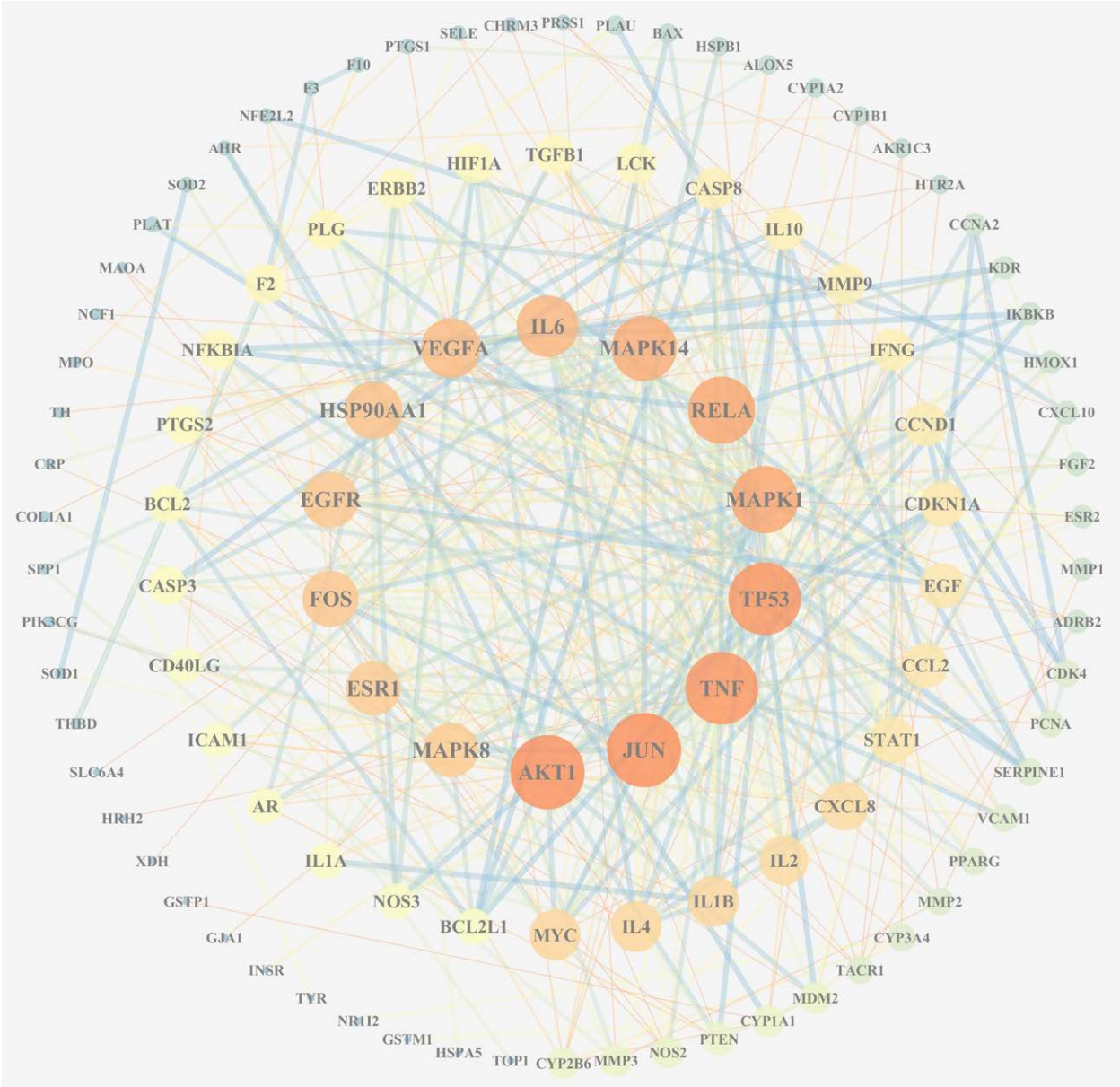

Figure 3. PPI network of intersection targets: the nodes from blue to orange, from small to large, indicating that the degree value increases gradually; the edges from orange to blue, from thin to thick, indicating that the combined score increases gradually. PPI = protein–protein interaction.

Table 2

Core targets of PPI network.

Target gene	Target protein	Degree	Betweenness centrality	Closeness centrality
AKT1	RAC-alpha serine/threonine-protein kinase	29	0.055047	0.468750
JUN	Transcription factor AP-1	29	0.105401	0.468750
TNF	Tumor necrosis factor	28	0.093889	0.483871
TP53	Cellular tumor antigen p53	28	0.071539	0.462555
MAPK1	Mitogen-activated protein kinase 1	25	0.035568	0.454545
RELA	Transcription factor p65	25	0.151709	0.486111
MAPK14	Mitogen-activated protein kinase 14	24	0.084774	0.435685

PPI = Protein–protein interaction.

proliferation of autoreactive T cells and subsets disorder of T cells are vital pathological mechanisms of SLE. In vitro studies have shown that luteolin can inhibit antigen-specific proliferation and interferon-γ production by murine and human autoimmune T cells.[28] Kaempferol could enhance the suppressive function of Treg cells by inhibiting FOXP3 phosphorylation; therefore, it might be effective for the prevention and treatment of inflammatory diseases such as SLE.[29] Besides, luteolin is reported to be beneficial for other autoimmune diseases. It can suppress the proliferation of peripheral blood mononuclear cells

Table 3

KEGG pathway enrichment analysis of intersection targets.

GO	Description	Count	LogP	Hits
hsa04151	PI3K-Akt signaling pathway	27	−24.37	AKT1, CCND1, BCL2, BCL2L1, CDK4 , CDKN1A, COL1A1, EGF, EGFR, FGF2, HSP90AA1, IKBKB, IL2, IL4, IL6, INSR, KDR, MDM2, MYC, NOS3, PIK3CG, MAPK1, PTEN, RELA, SPP1, TP53, VEGFA
hsa04657	IL-17 signaling pathway	23	−32.69	CASP3, CASP8, MAPK14, FOS, HSP90AA1, IFNG, IKBKB, IL1B, IL4, IL6, CXCL8, CXCL10, JUN, MMP1, MMP3, MMP9, NFKBIA, MAPK1, MAPK8, PTGS2, RELA, CCL2, TNF
hsa04668	TNF signaling pathway	22	−29.20	AKT1, CASP3, CASP8, MAPK14, FOS, ICAM1, IKBKB, IL1B, IL6, CXCL10, JUN, MMP3, MMP9, NFKBIA, MAPK1, MAPK8, PTGS2, RELA, CCL2, SELE, TNF, VCAM1
hsa04659	Th17 cell differentiation	19	−23.99	AHR, MAPK14, FOS, HIF1A, HSP90AA1, IFNG, IKBKB, IL1B, IL2, IL4, IL6, JUN, LCK, NFKBIA, MAPK1, MAPK8, RELA, STAT1, TGFB1
hsa04010	MAPK signaling pathway	19	−16.63	AKT1, CASP3, MAPK14, EGF, EGFR, FGF2, FOS, HSPB1, IKBKB, IL1A, IL1B, JUN, MYC, MAPK1, MAPK8, RELA, TGFB1, TNF, TP53
hsa04066	HIF-1 signaling pathway	17	−21.05	AKT1, BCL2, CDKN1A, EGF, EGFR, ERBB2, HIF1A, HMOX1, IFNG, IL6, INSR, NOS2, NOS3, SERPINE1, MAPK1, RELA, VEGFA
hsa04620	Toll-like receptor signaling pathway	17	−20.82	AKT1, CASP8, MAPK14, FOS, IKBKB, IL1B, IL6, CXCL8, CXCL10, JUN, NFKBIA, MAPK1, MAPK8, RELA, SPP1, STAT1, TNF
hsa04621	NOD-like receptor signaling pathway	17	−17.1	BCL2, BCL2L1, CASP8, MAPK14, HSP90AA1, IKBKB, IL1B, IL6, CXCL8, JUN, NFKBIA, MAPK1, MAPK8, RELA, CCL2, STAT1, TNF
hsa04060	Cytokine-cytokine receptor interaction	17	−13.69	CD40LG, EGF, EGFR, IFNG, IL1A, IL1B, IL2, IL4, IL6, CXCL8, IL10, CXCL10, KDR, CCL2, TGFB1, TNF, VEGFA
hsa04660	T cell receptor signaling pathway	16	−19.24	AKT1, CD40LG, CDK4, MAPK14, FOS, IFNG, IKBKB, IL2, IL4, IL10, JUN, LCK, NFKBIA, MAPK1, RELA, TNF
hsa04068	FoxO signaling pathway	16	−17.44	AKT1, CCND1, CDKN1A, MAPK14, EGF, EGFR, IKBKB, IL6, IL10, INSR, MDM2, MAPK1, MAPK8, PTEN, SOD2, TGFB1
hsa04210	Apoptosis	15	−15.64	AKT1, BAX, BCL2, BCL2L1, CASP3, CASP8, FOS, IKBKB, JUN, NFKBIA, MAPK1, MAPK8, RELA, TNF, TP53
hsa04064	NF-kappa B signaling pathway	14	−16.53	BCL2, BCL2L1, CD40LG, ICAM1, IKBKB, IL1B, CXCL8, LCK, NFKBIA, PLAU, PTGS2, RELA, TNF, VCAM1
hsa04658	Th1 and Th2 cell differentiation	13	−15.13	MAPK14, FOS, IFNG, IKBKB, IL2, IL4, JUN, LCK, NFKBIA, MAPK1, MAPK8, RELA, STAT1
hsa04630	Jak-STAT signaling pathway	12	−10.79	AKT1, CCND1, BCL2, BCL2L1, CDKN1A, IFNG, IL2, IL4, IL6, IL10, MYC, STAT1
hsa04014	Ras signaling pathway	12	−8.90	AKT1, BCL2L1, EGF, EGFR, FGF2, IKBKB, INSR, KDR, MAPK1, MAPK8, RELA, VEGFA
hsa04915	Estrogen signaling pathway	11	−11.73	AKT1, EGFR, ESR1, ESR2, FOS, HSP90AA1, JUN, MMP2, MMP9, NOS3, MAPK1
hsa04115	p53 signaling pathway	10	−11.85	BAX, CCND1, CASP3, CASP8, CDK4, CDKN1A, MDM2, SERPINE1, PTEN, TP53
hsa04622	RIG-I-like receptor signaling pathway	9	−10.23	CASP8, MAPK14, IKBKB, CXCL8, CXCL10, NFKBIA, MAPK8, RELA, TNF
hsa04370	VEGF signaling pathway	8	−9.34	AKT1, MAPK14, HSPB1, KDR, NOS3, MAPK1, PTGS2, VEGFA

GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, TNF = tumor necrosis factor.

and the secretion of IL-1β, TNF-α and matrix metalloprotein-9 in multiple sclerosis patients in a dose-dependent manner[30]; up-regulate the expression of ciliary neurotrophic factor and cyclic adenosine monophosphate in autoimmune encephalomyelitis rats to increase the antioxidant capacity of brain tissue.[31] Quercetin shows renoprotective effects in the pristane-induced lupus nephritis mice model.[32] It could ameliorate the symptoms of lupus nephritis in the cGVHD mouse model by inhibiting the activation of $CD4^+$ T cells and the pro-inflammatory response of macrophages.[33] In addition, its renoprotective effects may also be due to the inhibition of nuclear factor-κB (NF-κB) signaling pathway, which could further reduce the expression of PTX3 and the excessive proliferation of mesangial cells.[34]

A total of 118 intersection targets between QH-BJ drug pair and SLE were obtained. Furthermore, we screened 7 core targets of the PPI network and 5 core targets of the component-target-pathway network, which were regarded as the core targets of QH-BJ drug pair for SLE. RELA, MAPK1, and MAPK14 occur in both networks. The majority of the 9 core targets are closely related to inflammation and immune disorders. For example, the protein encoded by RELA gene is transcription factor p65, a subunit of NF-κB complex. MAPK (mitogen-activated protein kinase) transduces signals from the cell surface to the nucleus. Both NF-κB signals and MAPK signals are key regulators of the immune system and inflammatory response.[35–37] Besides, COX-2 and PTGS2, respectively, are the action target and the downstream target of non-steroidal anti-inflammatory drugs which are widely used in SLE. The pivotal active components and core targets have been further verified by molecular docking which shows that the 3 ligand molecules (luteolin, quercetin, and kaempferol) all can spontaneously bind to the nine receptor target proteins.

KEGG pathway enrichment analysis indicates that PI3K/Akt signal pathway, IL-17 signal pathway, and TNF signal pathway may be the main pathways underlying QH-BJ drug pair. PI3K/Akt signaling pathway plays a central role in multiple functions of mammalian cells, such as cell growth, migration, proliferation, and metabolism.[38,39] Studies have shown that PI3K/Akt pathway is involved in the regulation of T and B lymphocyte activation.[40,41] M2a macrophages, a kind of anti-inflammatory phenotype, have been reported to be helpful to alleviate SLE and the activation of PI3K/Akt signals could promote macrophage polarization from M2b (proinflammatory phenotype) to M2a.[42,43] Disruption of IL-17 homeostasis has been associated with the development and progression of rheumatic diseases, and blocking IL-17 might be a promising strategy in the treatment of systemic rheumatic diseases such as SLE.[44] The level of IL-17 in the circulation of SLE patients increased, and IL-17-producing cells were observed in affected organs; further research showed that IL-17 signals could promote the production of autoantibodies, the deposition of immune complexes, and the activation of complements.[45,46] TNF is an important inflammation marker in SLE, and involves in the pathogenesis of SLE.[47,48] Anti-TNF biologics have achieved great success in the treatment of autoimmune diseases such as rheumatic arthritis.[49] Therefore, TNF might be a potential treatment target for SLE.[50]

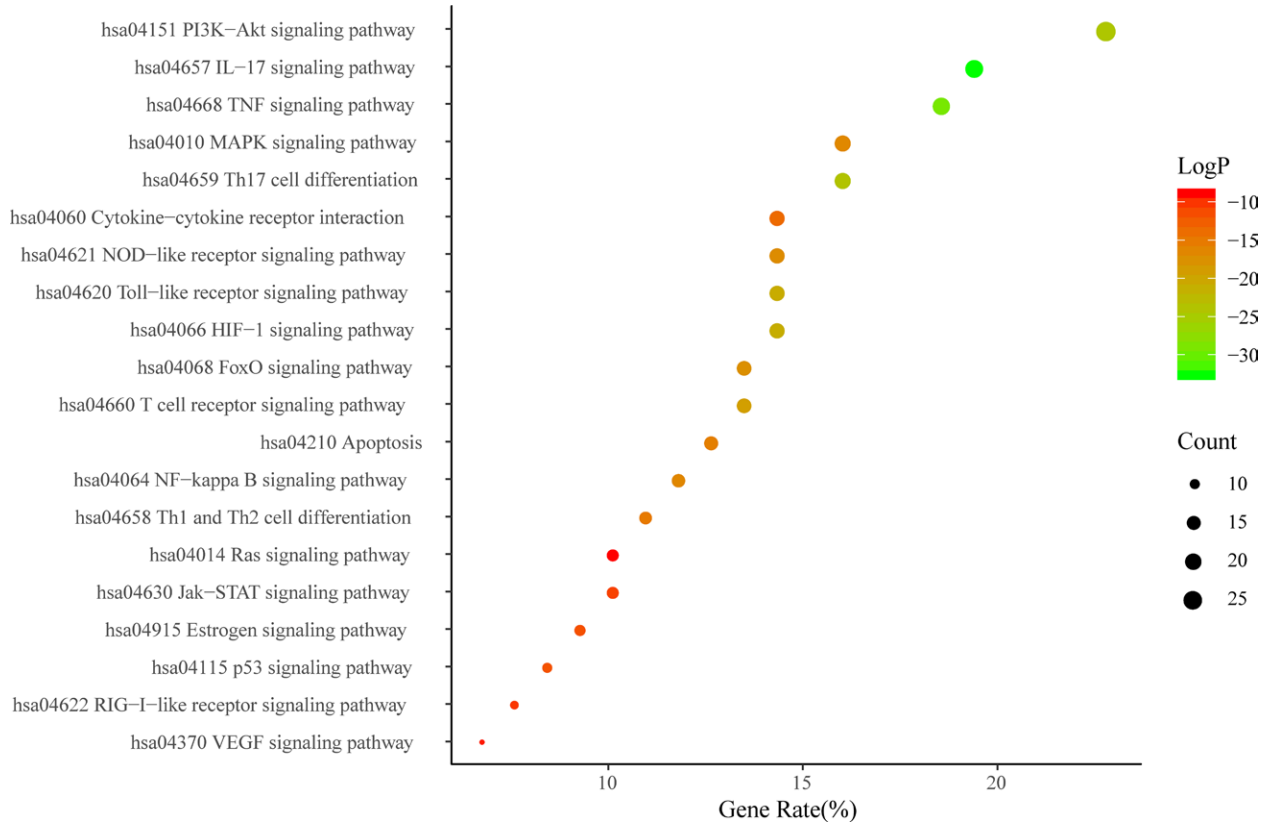

Figure 4. KEGG pathway enrichment analysis of intersection targets. KEGG = Kyoto Encyclopedia of Genes and Genomes.

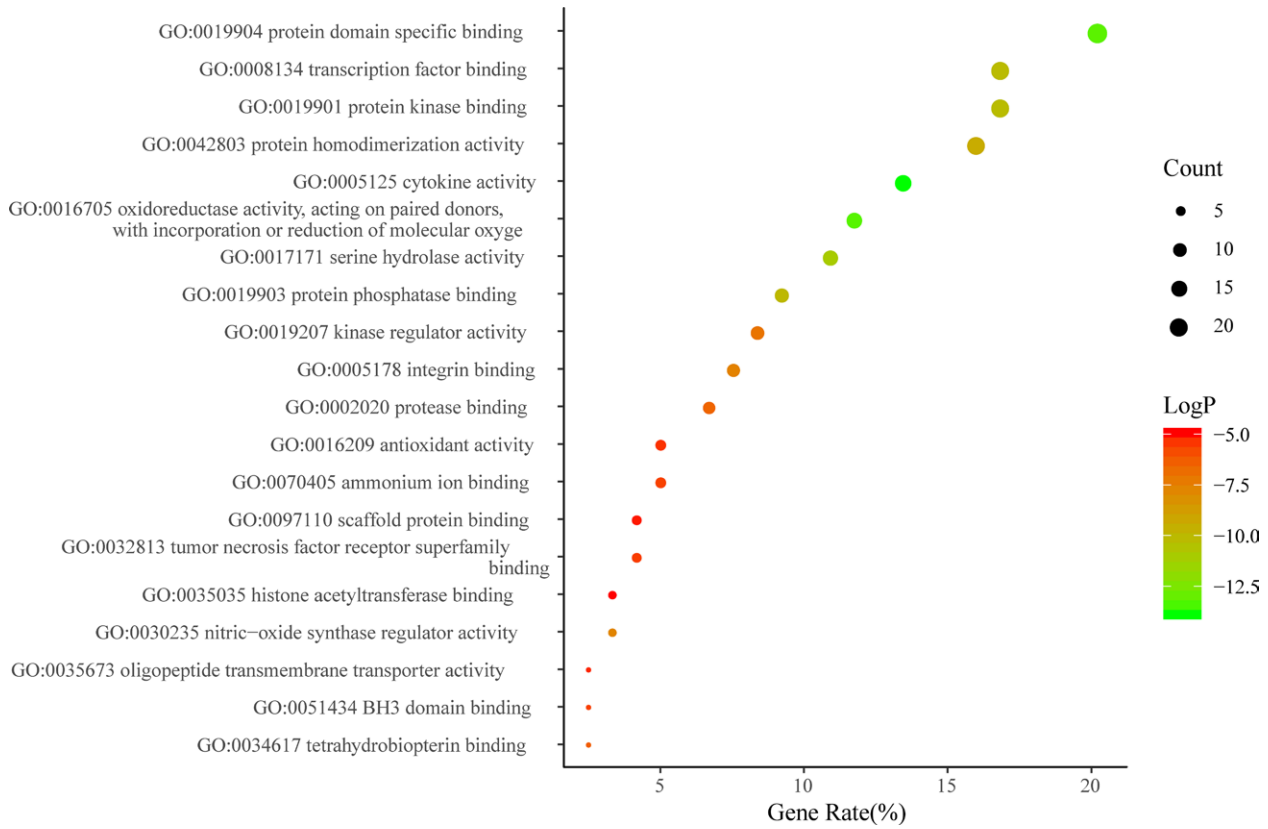

Figure 5. GO-MF analysis of intersection targets. GO = gene ontology, MF = molecular functions.

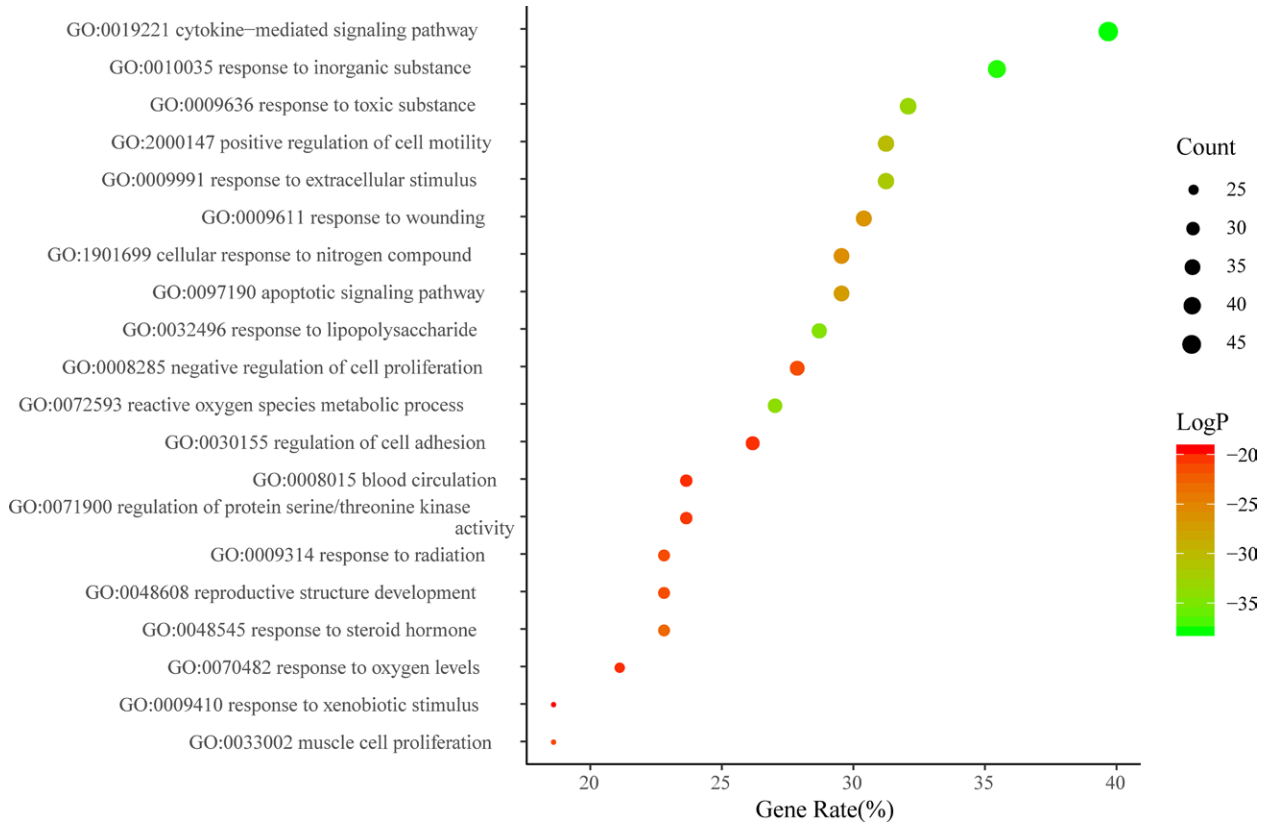

Figure 6. GO-BP analysis of intersection targets. BP = biological processes, GO = gene ontology.

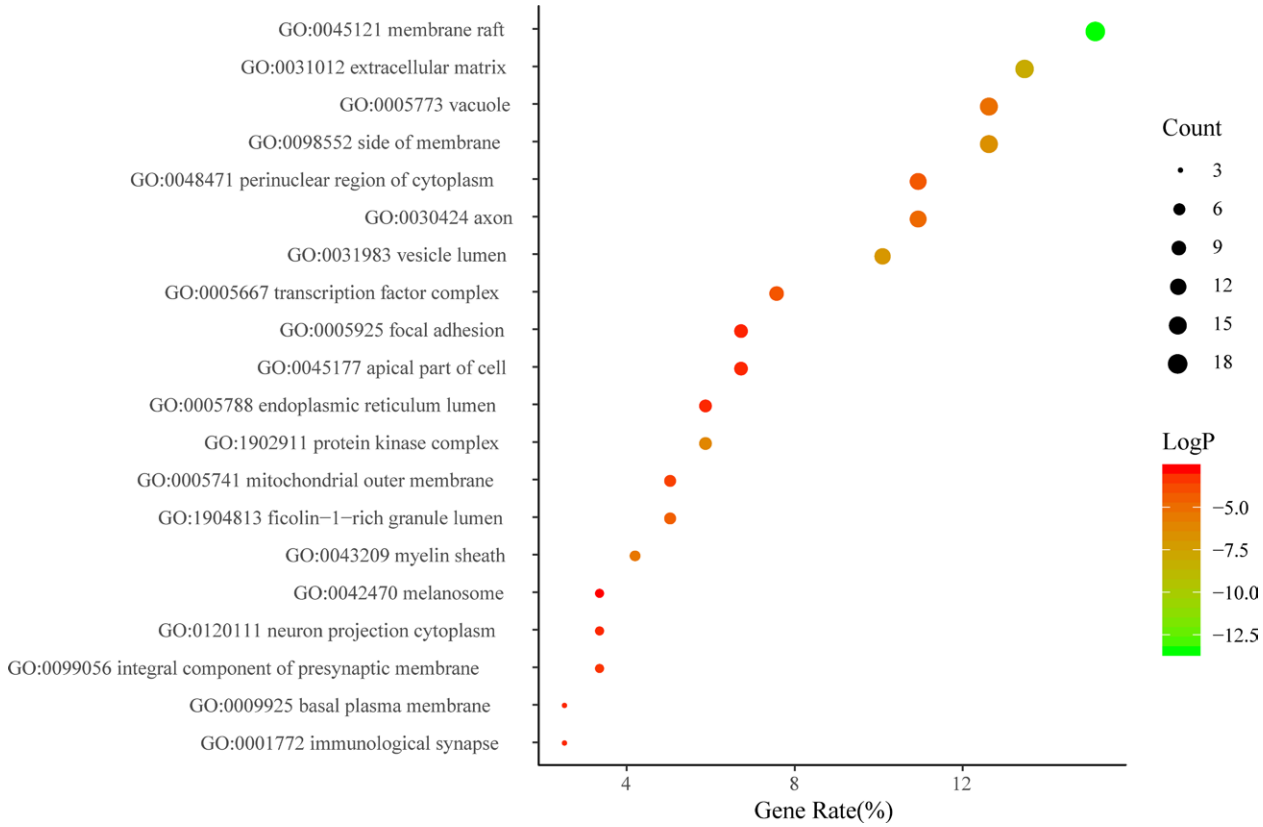

Figure 7. GO-CC analysis of intersection targets. CC = cellular components, GO = gene ontology.

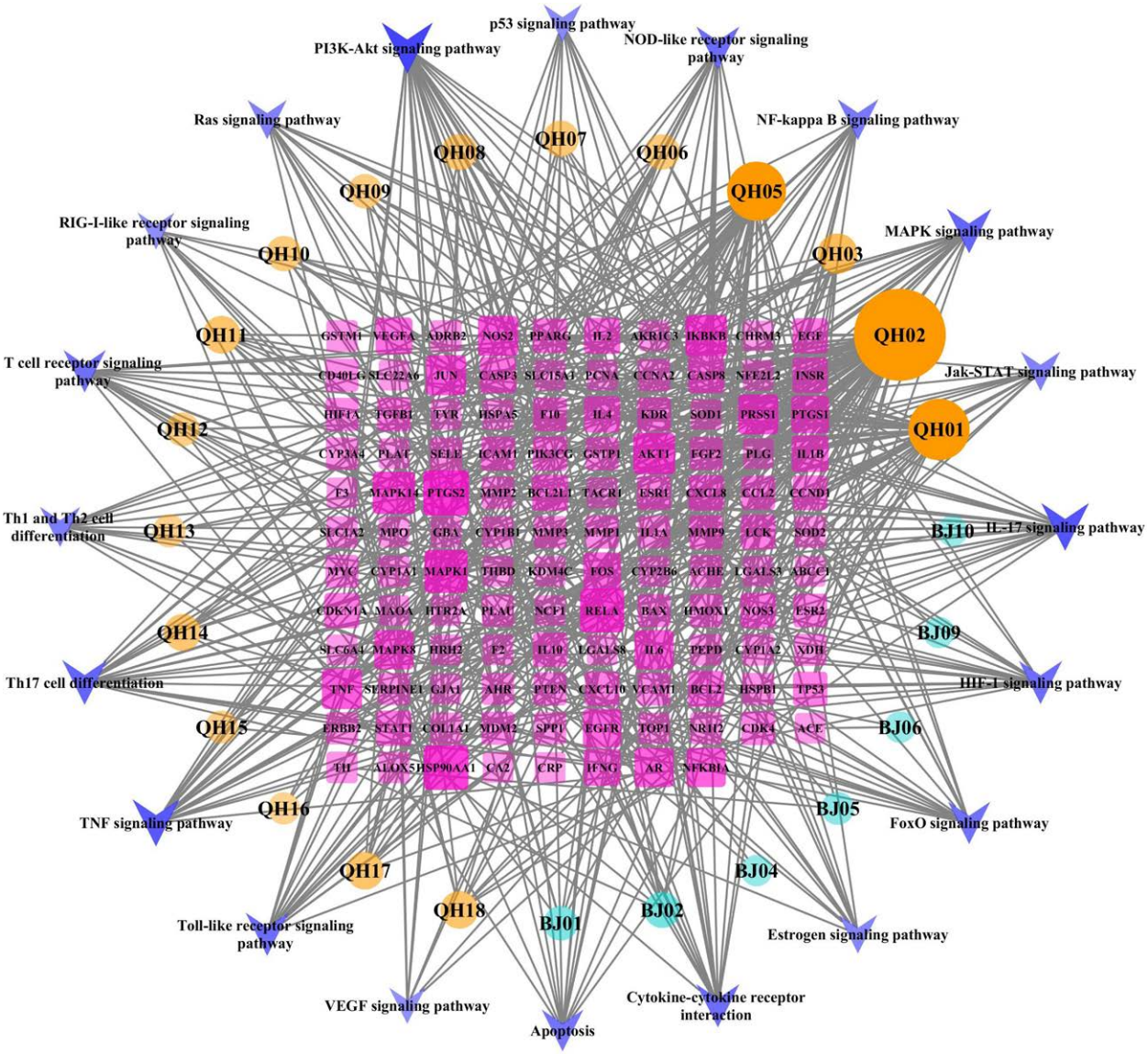

Figure 8. Predicted component-target-pathway network of QH-BJ drug pair for the treatment of SLE: circle represents components, square represents targets, and the V shape represents pathways; the area and color transparency of the nodes represent their degree value, and the larger the area and the darker the color, the greater the degree value of the node. BJ = Biejia, QH = Qinghao, SLE = systemic lupus erythematosus.

Table 4

Core active compounds of component-target-pathway network.

ID	Molecule ID	Molecule name	Degree	Betweenness centrality	Closeness centrality
QH01	MOL000006	luteolin	40	0.115632	0.452096
QH02	MOL000098	quercetin	79	0.434986	0.589844
QH05	MOL000422	kaempferol	37	0.0819	0.424157

Table 5

Core targets of component-target-pathway network.

Target gene	Target protein	Degree	Betweenness centrality	Closeness centrality
PTGS2	Prostaglandin G/H synthase 2	20	0.070394	0.460366
HSP90AA1	Heat shock protein HSP 90-alpha	19	0.070851	0.460366
RELA	Transcription factor p65	18	0.031556	0.446746
MAPK1	Mitogen-activated protein kinase 1	17	0.018086	0.44152
MAPK14	Mitogen-activated protein kinase 14	16	0.022039	0.3775

5. Conclusion

The study indicates that QH-BJ drug pair might play a role in the treatment of SLE through multi-components, multi-targets, and multi-pathways, providing strategies for further experimental verification.

Author contributions

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Supervision: Yongsheng Fan, Qin Zhang, Kepeng Yang.

Table 6

Molecular docking of core active compounds and core targets.

	Binding affinity		
Targets	**Luteolin**	**Quercetin**	**Kaempferol**
PTGS2	−9.4	−9.7	−9.4
HSP90AA1	−9.3	−8.9	−8.8
RELA	−7.6	−7.4	−7.4
MAPK1	−7.6	−7.8	−7.3
MAPK14	−9.1	−8	−9.3
AKT1	−10	−10.4	−9.8
JUN	−6	−6	−5.9
TNF	−9	−9	−8.8
TP53	−7.8	−8	−7.9

Validation: Shan Wu.
Visualization: Lina Ji, Shan Wu, Sijia Fang.
Writing – original draft: Ziyu Song, Sijia Fang.
Writing – review & editing: Ziyu Song.

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