

Integrated bioinformatics and network pharmacology to identify the therapeutic target and molecular mechanisms of *Scutellaria barbata* plus *Hedyotis diffusa* herb pair on esophageal squamous cell carcinoma

Xiaoliang Xing

Hunan University of Medicine

Chaoqun Xing

The First Affiliated Hospital of Hunan University of Medicine

Xuemei Zhang

Hunan University of Medicine

Zhiyong Yao (✉ zhiyongyaohnum@163.com)

Hunan University of Medicine

Research Article

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Abstract

Objective: This study aims to systematically investigate the therapeutic targets and molecular mechanisms of *Scutellaria barbata* plus *Hedyotis diffusa* herb pair (SBHD) on esophageal squamous cell carcinoma (ESCC) based on GEO gene microarray combined with network pharmacology and molecular docking technology.

Methods: The active components and effective targets of SBHD were retrieved and downloaded from the TCMSD database, and the differentially expressed genes (DEGs) of ESCC were retrieved and downloaded from the GEO database. The intersection targets between medicine target genes and disease target genes were screened by drawing Venn diagram. Bioinformatics tools such as R language, Cytoscape software, STRING platform, and DAVID platform, were applied to perform active components-targets regulatory network analysis, PPI network analysis, and GO and KEGG pathway enrichment analysis. Molecular docking was performed to validate the interaction between the core active components and the key target genes by AutoDock Vina tools.

Results: A total of 33 main active components were predicted from herb pair, and 28 intersection targets were screened from 105 medicine target genes and 4064 disease target genes. A topological analysis of the active components-targets regulatory network and PPI network revealed 5 core ingredients and 6 key targets for SBHD treating ESCC, respectively. KEGG enrichment analysis found that SBHD could affect cellular senescence, hepatitis B, MAPK signaling pathway, proteoglycans in cancer and apoptosis in ESCC. Molecular docking found that the 5 core active compounds had good binding properties with the 6 key therapeutic targets.

Conclusion: The therapeutic effects of SBHD on ESCC might be related to the active components including quercetin, baicalein, luteolin, stigmasterol and wogonin, which intervened with the key targets including IL6, CASP3, MYC, AR, CAV1 and RUNX2, and the signaling pathway including cellular senescence, hepatitis B, MAPK signaling pathway, proteoglycans in cancer and apoptosis.

1. Introduction

Esophageal cancer is the seventh most common incident cancer and the sixth leading cause of cancer mortality in the world ^[1]. About 572,000 new cases of esophageal cancer are diagnosed each year and more than 509,000 deaths are estimated to die from it ^[2]. Most patients with esophageal cancer are in an advanced stage at diagnosis, even with active surgery and adjuvant therapy, the prognosis is still poor ^[3]. Esophageal squamous cell carcinoma (ESCC) is the predominant histologic type of esophageal cancer accounting for 84% of all cases, followed by esophageal adenocarcinoma accounting for 15% of all cases ^[4]. Currently, there are no effective prophylaxis and therapeutic strategies for ESCC ^[5]. Moreover, due to frequent recurrence after surgery, and the effect is not satisfactory after chemotherapy or radiotherapy, their overall 5-year survival rate is < 20% ^[6]. Therefore, it is very urgent that the development of new therapeutic drugs or the search of effective therapeutic targets for this lethal disease.

Traditional Chinese Medicine (TCM) originated in ancient China. In recent years, there is an increasing interest in using TCM and their patents for the treatment of cancers [7]. Compared with Western medicine, TCM has the characteristics of long-term administration, fewer side effects, and less drug resistance [8, 9]. In particular, the combined application of Chinese Medicine and Western Medicine has remarkable clinical effect in treatment of cancer [10]. TCM not only can directly eliminate carcinogenic [11], but also can improve the physique to make the body no longer suitable for tumor growth [12, 13]. TCM theory believes that the key pathological factors for esophageal cancer are deficiency (xu), stasis (yu), heat (re), and poison (du), and the main therapies methods should follow these principles, such as replenishing qi, promoting blood circulation, invigorating the spleen and kidney, regulating qi and phlegm, and clearing heat and toxic substances [14].

Scutellaria barbata (SB) and *Hedyotis diffusa* (HD) are both heat-clearing and detoxifying natural herbal medicines according to TCM theory and always used together to treat numerous cancers [15]. It is reported that the combined use of the two herbs is not simply superimposed, and their combined application is more effective than a single drug [16]. Modern pharmacological studies found that SB plus HD herb pair (SBHD) could induce cancer apoptosis, inhibit cancer cell proliferation, and suppresses cancer cells migration [17–20]. In recent years, the two herbs and their extracts have been widely used in the treatment of esophageal cancer and have shown remarkable effects [21–23]. However, the detailed anticancer effect, underlying therapeutic targets and molecular mechanisms of SBHD on ESCC remain unclear.

Network pharmacology, an integrated biomedical concept, which is based on bioinformatics, system biology and modern pharmacology, affords a novel network mode of “multiple ingredients, multiple targets, and multiple mechanisms” [24]. In this study, a search of TCMSP databases was performed to identify the active components and targets for drugs, combined with the analysis of multiple GEO gene microarray to obtain disease related targets, and matched them to obtain potential therapeutic targets of SBHD on ESCC. Subsequently, active components-targets network and PPI network were constructed, and core active compounds and key targets were screened according to topological parameters, respectively. GO and KEGG enrichment analyses were performed to explore the molecular mechanisms. Finally, molecular docking technology was used to verify the interaction between core active compounds and key targets. A Workflow diagram of this research is presented in Fig. 1.

2. Materials And Methods

2.1 Active compounds and effective targets of SBHD

Using “*Scutellaria barbata*” and “*Hedyotis diffusa*” as searching keywords in the TCMSP database (<https://old.tcmsp-e.com/tcmsp.php>), the active compounds and related targets of SBHD were identified [25]. The main active components were then filtered according to the optimal toxicity kinetic ADME rules [Oral Bioavailability (OB) $\geq$ 30%, Drug-Like (DL) $\geq$ 0.18] [26]; At the same time, the effective targets

associated with active compounds were retrieved from the TCMSP database, and the retrieved targets were converted into standardized gene symbol by the UniProt database (<https://www.uniprot.org>)^[27].

2.2 DEGs of ESCC

Expression profiling data from GSE17351, GSE26886, and GSE161533 were downloaded from the NCBI GEO gene microarray database (<https://www.ncbi.nlm.nih.gov/geo/>) based on the GPL570 microarray platform^[28]. The GSE17351 datasets contained 5 normal samples and 5 tumor samples, the GSE26886 datasets contained 19 normal samples and 9 tumor samples, and the GSE161533 datasets contained 28 normal samples and 28 tumor samples, respectively. DEGs between ESCC patients with normal tissues and tumor tissues were obtained using the “limma” package of R 4.2.1 software according to the screening conditions of “adjusted P value < 0.05” and “ $|\log_2FC| > 1$ ”^[29]. The volcano plot and heat map of the DEGs from three gene chips were then plotted using the “ggplot2” and “pheatmap” package in the R software. Finally, we combined the DEGs in the three datasets, deleted the duplicate values, and established the targets database related to ESCC.

2.3 “Active compounds-targets” regulatory network

The potential active compounds targets and potential disease targets were taken intersection by the “VennDiagram” package in the R software, and defined these intersection targets as potential therapeutic targets of SBHD on ESCC. These intersection targets and their corresponding active compounds were introduced into the Cytoscape software to construct the “active compounds- targets” regulatory network. The “Analyze Network” plug-in in the Cytoscape software was then used to calculate and sort the topological parameters (Degree) of the network^[30], and then obtained the core active compounds related to SBHD treating ESCC.

2.4 PPI network and core sub network

The potential therapeutic targets of SBHD on ESCC were then uploaded into the STRING online platform (<https://cn.string-db.org/>) to construct the PPI network^[31]. The species was set as “Homo sapiens”, and the minimum required interaction score was set as “medium confidence (0.400)”^[32]. The PPI network file was then loaded into the Cytoscape software for visualization, and the “CytoHubba” plug-in was used to calculate three topological parameters including Degree Centrality (DC), Closeness Centrality (CC), and Betweenness Centrality (BC)^[33]. These three topological parameters, a higher value represents greater importance of the node in the merged network^[34]. Subsequently, these screened hub genes were imported into the Cytoscape software, and constructed the PPI core sub network.

2.5 GO and KEGG pathway enrichment

The potential therapeutic targets of SBHD on ESCC were imported into the DAVID data online platform (<https://david.ncifcrf.gov/>), the “Homo sapiens” species were selected for GO and KEGG pathway enrichment analysis^[35], and “ P value < 0.05” indicates significant differences^[36]. Select GO terms and KEGG pathways in the top 15 of the P value and use the bioinformatics online platform

(<https://www.bioinformatics.com.cn/>) to visualize these enrichment analysis results. Subsequently, the network diagram of the major KEGG pathways was mapped using KEGG Mapper platform (<https://www.genome.jp/kegg/mapper/>)^[37].

2.6 Molecular Docking

Molecular docking was utilized to validate the association between the core active compounds and the key targets. The 2D structure of the core active compounds were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>)^[38], which imported into the Chem3D 20.0 software to draw 3D structure diagrams, and optimize energy (MM2 calculation method) and save them in mol2 format. The 3D structure of the key target proteins were obtained from the RCSB PDB database (<https://www.rcsb.org/>)^[39], and then PyMol 2.6.0 software was used to remove water and natural ligands from the protein. AutoDock Vina tools was used to finish the molecular docking between ligand and receptor^[40], and Pymol software was used to visualize the docking conformations. According to previous studies, two molecules have excellent binding activity when the binding energy is less than -5.0 kcal/mol^[41].

3. Results

3.1 Screening of active compounds and effective targets of SBHD

SB contains 94 active components and HD contains 37 active components in TCMSP database. After screening based on the “OB > 30%” and “DL > 0.18” criteria, 33 main active compounds were identified refer to 29 active compounds in SB, 7 active compounds in HD, and 3 common active compounds (MOL000098, MOL000449, and MOL000358) in the two herbs (Table 1). A total of 237 SBHD-related targets were found from the TCMSP database and standardized using the UniProt database. Finally, a total of 105 effective targets related to SBHD were obtained after removing the chemical compounds that lacked potential target information.

Table 1
Main active compounds of SBHD

ID	Compound	OB (%)	DL	Herb
MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	74.24	0.26	SB
MOL005190	eriodictyol	71.79	0.24	SB
MOL012248	5-hydroxy-7,8-dimethoxy-2-(4-methoxyphenyl) chromone	65.82	0.33	SB
MOL002915	Salvigenin	49.07	0.33	SB
MOL000351	Rhamnazin	47.14	0.34	SB
MOL012269	Stigmasta-5,22-dien-3-ol-acetate	46.44	0.86	SB
MOL000098	quercetin	46.43	0.28	SB/HD
MOL012270	Stigmastan-3,5,22-triene	45.03	0.71	SB
MOL008206	Moslosooflavone	44.09	0.25	SB
MOL001659	Poriferasterol	43.83	0.76	HD
MOL000449	Stigmasterol	43.83	0.76	SB/HD
MOL012250	7-hydroxy-5,8-dimethoxy-2-phenyl-chromone	43.72	0.25	SB
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21	SB
MOL001973	Sitosteryl acetate	40.39	0.85	SB
MOL002776	Baicalin	40.12	0.75	SB
MOL012252	9,19-cyclolanost-24-en-3-ol	38.69	0.78	SB
MOL012266	rivularin	37.94	0.37	SB
MOL000953	CLR	37.87	0.68	SB
MOL001670	2-methoxy-3-methyl-9,10-anthraquinone	37.83	0.21	HD
MOL012254	campesterol	37.58	0.71	SB
MOL012251	Chrysin-5-methylether	37.27	0.2	SB
MOL000358	beta-sitosterol	36.91	0.75	SB/HD
MOL005869	daucostero_qt	36.91	0.75	SB
MOL000359	sitosterol	36.91	0.75	SB
MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	36.63	0.27	SB
MOL000006	luteolin	36.16	0.25	SB

ID	Compound	OB (%)	DL	Herb
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	SB
MOL001646	2,3-dimethoxy-6-methyanthraquinone	34.86	0.26	HD
MOL002714	baicalein	33.52	0.21	SB
MOL002719	6-Hydroxynaringenin	33.23	0.24	SB
MOL001663	(4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydronicene-4a-carboxylic acid	32.03	0.76	HD
MOL001735	Dinatin	30.97	0.27	SB
MOL000173	Wogonin	30.68	0.23	SB

3.2 Identification of DEGs of ESCC

With “adjusted P value < 0.05” and “ $|\log_2FC| > 1$ ” as screening conditions, the differential expression analysis of the three gene chips (GSE17351, GSE26886, and GSE161533) identified 645, 3446, and 915 DEGs in ESCC tumor tissue compared with normal esophagus squamous epithelial tissue, respectively. Subsequently, the DEGs from the three data sets were combined, removed duplications, and a total of 4064 potential target genes may be related to ESCC were obtained. The DEGs associated with ESCC analyzed from the three data sets were displayed by

volcano plot and heat map, respectively (Fig. 2).

3.3 Construction of “active compounds-targets” regulatory network

The potential drug targets were matched with potential disease targets, resulting in the selection of 28 potential therapeutic targets of SBHD on ESCC (Fig. 3). The “active compounds-targets” regulatory network was constructed to demonstrate the relationship between active compounds and intersection targets including 52 nodes (24 active compounds and 28 potential therapeutic targets) and 78 edges (Fig. 4). As shown in the figure, quercetin, baicalein, luteolin, stigmasterol, and wogonin have more corresponding therapeutic targets, with 22, 7, 5, 5, and 5 target genes, respectively. The 5 active compounds may be the core active ingredients of SBHD treating ESCC.

3.4 Construction of PPI network and core sub network

The 28 potential therapeutic targets of SBHD on ESCC into STRING database, removed isolated nodes, and then successfully constructed a PPI network by Cytoscape software (Fig. 5A). As shown in the figure, the PPI network comprised 26 nodes and 76 edges. To screen out key target genes, the three parameters

(DC, CC, and BC) of each node were calculated using the “Cytohubba” plug-in in Cytoscape software. The DC, CC, and BC of 6 nodes named IL6, CASP3, MYC, AR, CAV1, and RUNX2, exceeded the average values of the topological parameters of the whole network (Table 2), demonstrating that these 6 hub genes might be the key targets of SBHD in treatment of ESCC (Fig. 5B).

Table 2
Topological parameters of 28 potential therapeutic targets.

Target	BC	CC	DC	Target	BC	CC	DC
IL6	254.8524	22.33333	20	FOSL1	0	14	4
CASP3	107.3024	20	15	CHEK1	48	13.5	4
MYC	64.66905	19.5	14	CYP1B1	0.4	13.66667	3
AR	25.35238	18.33333	12	NR3C2	0	13.41667	3
CAV1	28.41667	17.66667	11	RASA1	0	12.83333	3
FOS	11.46667	16.83333	9	AKR1B1	0	13.16667	2
IGFBP3	9.2	15.66667	7	SELE	0	13.16667	2
HSPB1	1.81905	15.66667	7	COL3A1	0	12.58333	2
RAF1	3.78571	15	7	CHRM3	0	12.08333	1
RUNX2	48	15.33333	6	DUOX2	0	12.08333	1
PLAU	2.26905	15.16667	6	PTGS1	0	12.08333	1
PARP1	8.46667	14.83333	5	PSMD3	0	9.75	1
AHR	2	14.66667	5	NUF2	0	9	1

3.5 GO and KEGG enrichment analysis

Through GO enrichment analysis, we obtained 616 GO items including 531 in biological process (BP), 46 in cellular component (CC), and 39 in molecular function (MF). The top 15 BP terms containing response to oxygen-containing compound, response to chemical, response to nitrogen compound, cellular response to nitrogen compound, response to organonitrogen compound, response to endogenous stimulus, response to ketone, response to hormone, response to organic substance, regulation of cell proliferation, cellular response to organonitrogen compound, response to organic cyclic compound, cellular response to chemical stimulus, intracellular signal transduction, and cellular response to endogenous stimulus (Fig. 6).

Through KEGG enrichment analysis, a total of 32 signaling pathways were obtained. The top 15 KEGG pathways mainly including Chemical carcinogenesis-receptor activation, Cellular senescence, Hepatitis B, MAPK signaling pathway, Colorectal cancer, Transcriptional misregulation in cancer, Kaposi sarcoma-

associated herpesvirus infection, IL-17 signaling pathway, Proteoglycans in cancer, AGE-RAGE signaling pathway in diabetic complications, Amoebiasis, Human T-cell leukemia virus 1 infection, TNF signaling pathway, Salmonella infection, and Apoptosis (Fig. 7). MAPK signaling pathway was then mapped, and enriched targets refer to MYC, RASA1, CASP3, HSPB1, FOS, and RAF1 (Fig. 8).

3.6 Molecular docking verification

6 key targets (IL6, CASP3, MYC, AR, CAV1, and RUNX2) were combined with 5 core active compounds (quercetin, baicalein, luteolin, stigmasterol, and wogonin) for molecular docking to evaluate active compounds and target genes binding potential. As a result, all receptor-ligand docking affinity scores were less than -5 kcal/mol, indicating that the binding activity between the receptor and ligand was good (Fig. 9). Among them, quercetin had the best docking activity with MYC, and their docking affinity scores was -9.5 kcal/mol. The 3D diagrams of molecular docking models were displayed using the PyMol software (Fig. 10). As shown in the figure, the potential binding sites of MYC and quercetin including DG-6, DG-7, DC-16, DG-15, and DG-14, and the average values of hydrogen bond distances were $2.5 \text{ \AA}$.

4. Discussion

The term “herb pair” refers to the combined use of two single herbs that are used to treat a specific disease in order to enhance clinical efficacy or minimize adverse effects^[42]. SB belongs to the labiaceae, and it is sour in taste and cold in nature^[43]. HD belongs to the genus Hedyotis of Rubiaceae, and it is bitter, light, and cold in nature^[44]. As a common herb pair, SBHD have the functions, such as clearing away heat and toxins, removing blood stasis, promoting blood circulation, diuresis, and detumescence, which can be used as clinical adjuvant therapy for a variety of cancers^[16, 45]. However, the therapeutic effects and specific mechanisms of SBHD on ESCC remain unclear.

In this study, network pharmacological was used to systematically study the active ingredients of SBHD in treatment of ESCC. The results indicate that quercetin, baicalein, luteolin, stigmasterol, and wogonin have more targets than other active compounds, which may be the core active components of SBHD treating of ESCC. Quercetin and stigmasterol are both the main active components shared by SB and OD, and the other three are the main active components of SB. It has been reported that a dietary pattern rich in quercetin may play a protective role in the development of ESCC^[46]. Zheng et al.^[47] found that quercetin could increase apoptotic effects for ESCC. Zhao et al.^[48] found that quercetin inhibited the formation of esophageal precancerous lesions, and the inhibition was associated with decreased inflammation and esophageal cancer cell proliferation. Baicalein, a natural flavonoid, not only could directly induce apoptosis, but also inhibits the progression and promotes radiosensitivity for ESCC^[49, 50]. Luteolin is also plant flavonoid which can inhibits cell proliferation and induces cell apoptosis for human ESCC^[51, 52]. Stigmasterol is a natural phytosterols, and it could increase dietary intake of stigmasterol were associated with a decreased risk of ESCC^[53]. Wogonin is a plant flavonoid extracted from roots, which has various anti-cancer effects^[54].

PPI core network analysis found that the 6 hub genes, such as IL6, CASP3, MYC, AR, CAV1, and RUNX2, which may be the key targets for SBHD treating ESCC. IL6, one of the major cytokines in the tumor microenvironment, and its over-expression has been reported in almost all types of tumors ^[55]. IL6's over-expression is also a key event for ESCC carcinogenesis ^[56]. Tong et al. ^[57] found that IL-6 was closely related to the pathological development of ESCC, and mainly involved in tumor tissue angiogenesis and endothelial cell formation. CASP3 is the main effector of apoptosis and involved in tumor development ^[58]. Zhang et al. ^[59] found that the decrease of CASP3 transcriptional activity and gene expression might inhibit apoptosis, and thus be associated with an increased risk of ESCC. Luo et al. ^[60] found that the decrease of CASP3 transcriptional activity could promote cell proliferation and inhibit apoptosis, thereby exhibited its oncogenic role in ESCC. MYC encodes a transcription factor involved in the regulation of cellular proliferation, differentiation and apoptosis ^[61]. Lian et al. ^[62] found that MYC expression in tumor tissues was obviously higher than the adjacent normal tissues, and might be a new diagnostic indicator of ESCC. Wang et al. ^[63] reported that MYC amplification induced cell cycle dysregulation is a common cause for ESCC, while targeting MYC could cause significant tumor suppression both in vitro and in vivo. AR was widely expressed in human several tissues, and plays an important role in many kinds of cancers ^[64]. Zhang et al. ^[65] found that the protein expression of AR is up-regulated in the ESCC tissue samples, and AR over expression induced increases in ESCC cell invasion and proliferation in vitro. CAV1 is one of the major structural proteins of caveolae, and its overexpression correlates with a poor prognosis and tumor progression in ESCC ^[66]. Several studies have also confirmed that CAV1 expression is closely related to ESCC, and it could be a powerful prognostic marker for patients with ESCC ^[67–69]. RUNX2 is related to a variety of tumors, particularly tumor invasion and metastasis ^[70]. It has been reported that RUNX2 was highly expressed in esophageal carcinoma tissues and cells, while knockdown of RUNX2 markedly suppressed tumor formation in vivo ^[71].

GO enrichment analysis showed that the biological processes related to SBHD in treatment of ESCC were mainly containing response to oxygen-containing compound, response to chemical, response to nitrogen compound, cellular response to nitrogen compound, response to organonitrogen compound, response to endogenous stimulus, response to ketone, response to hormone, response to organic substance, regulation of cell proliferation, cellular response to organonitrogen compound, response to organic cyclic compound, cellular response to chemical stimulus, intracellular signal transduction, and cellular response to endogenous stimulus. The KEGG pathways were mainly involved chemical carcinogenesis-receptor activation, cellular senescence, hepatitis B, MAPK signaling pathway, colorectal cancer, transcriptional misregulation in cancer, kaposi sarcoma-associated herpesvirus infection, IL-17 signaling pathway, proteoglycans in cancer, AGE-RAGE signaling pathway in diabetic complications, amoebiasis, human T-cell leukemia virus 1 infection, TNF signaling pathway, salmonella infection, and apoptosis. The studies have shown that these signaling pathways play an important role in the occurrence and development of ESCC, such as cellular senescence ^[72], hepatitis B ^[73], MAPK signaling pathway ^[74], proteoglycans in cancer ^[75], and apoptosis ^[76]. The study results suggest that SBHD may play a therapeutic role in ESCC through the above pathways.

Molecular docking reveals the interaction between the components of the network, thus improving the accuracy of the network^[77]. In this study, the key target protein of SBHD on ESCC was docked with core compounds. The results showed that the 5 core active components (quercetin, baicalein, luteolin, stigmasterol, and wogonin) had good binding properties with the 6 key therapeutic targets (IL6, CASP3, MYC, AR, CAV1, and RUNX2) related to ESCC, which further verified the accuracy of target prediction by network pharmacology. These data may be of great significance for further experimental study to determine the molecular mechanisms of SBHD on ESCC and the development of anti-tumor natural herbal medicines.

5. Conclusion

In summary, this research mainly adopted network pharmacology method combined with GEO differential expression analysis and molecular docking technology to systematically elaborate the therapeutic target and molecular mechanisms of SBHD on ESCC. The results indicated that quercetin, baicalein, luteolin, stigmasterol, and wogonin are the core active components of SBHD, which may generate therapeutic effects on ESCC through the key targets named IL6, CASP3, MYC, AR, CAV1, and RUNX2, and the major signaling pathways such as cellular senescence, hepatitis B, MAPK signaling pathway, proteoglycans in cancer and apoptosis. This study revealed the material basis and molecular mechanism for SBHD in treatment of ESCC, which provided a direction for further experimental research.

Declarations

Ethics approval and consent to participate

GEO databases belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study is available.

Competing interests

The authors declare no competing interests.

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Authors' contributions

X.X. and C.X. designed the study. X.X. and X.Z. collected data. X.X. and Z.Y. performed the data analysis and wrote the article.

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Figures

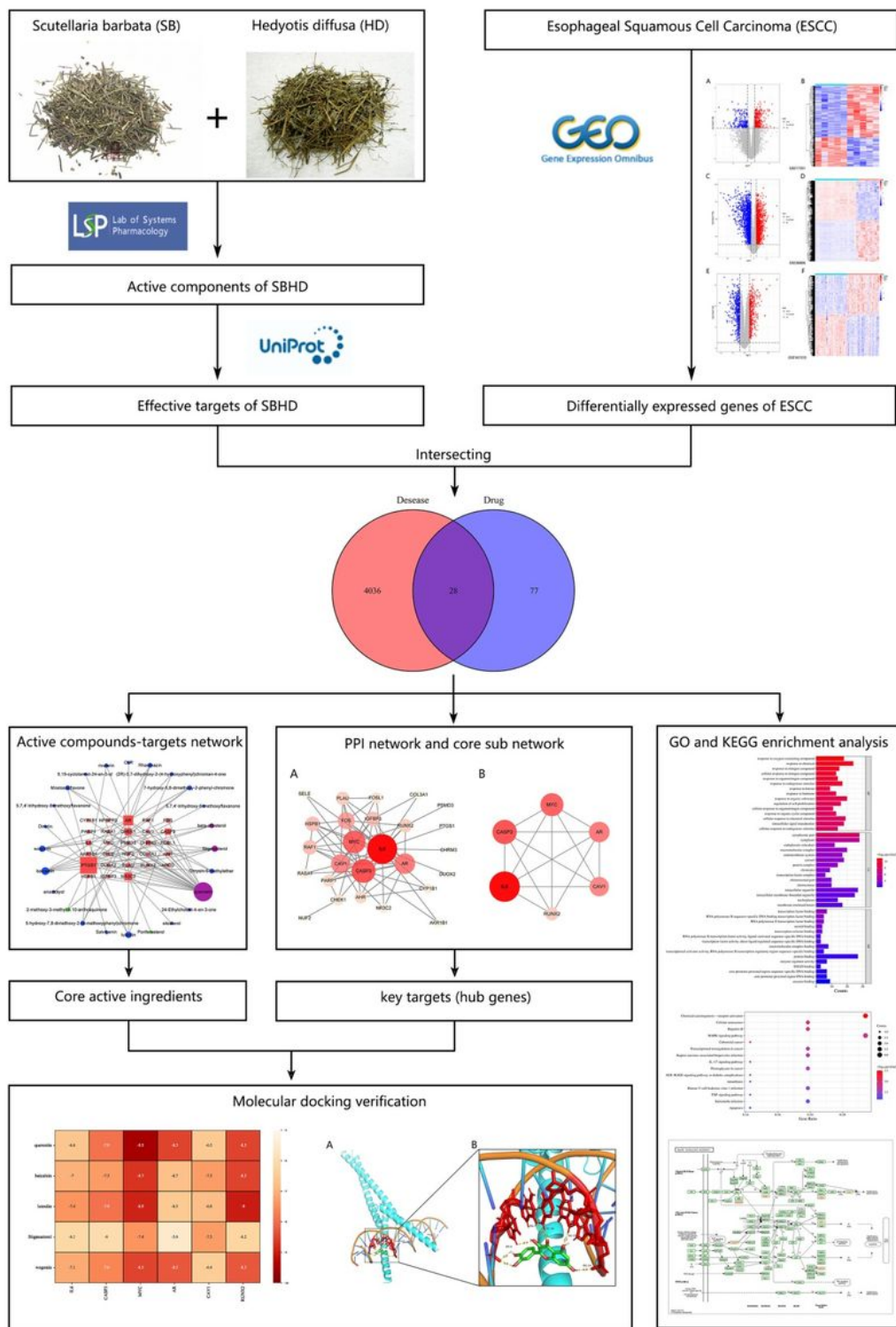


Figure 1

Workflow diagram of the bioinformatics and network pharmacology.

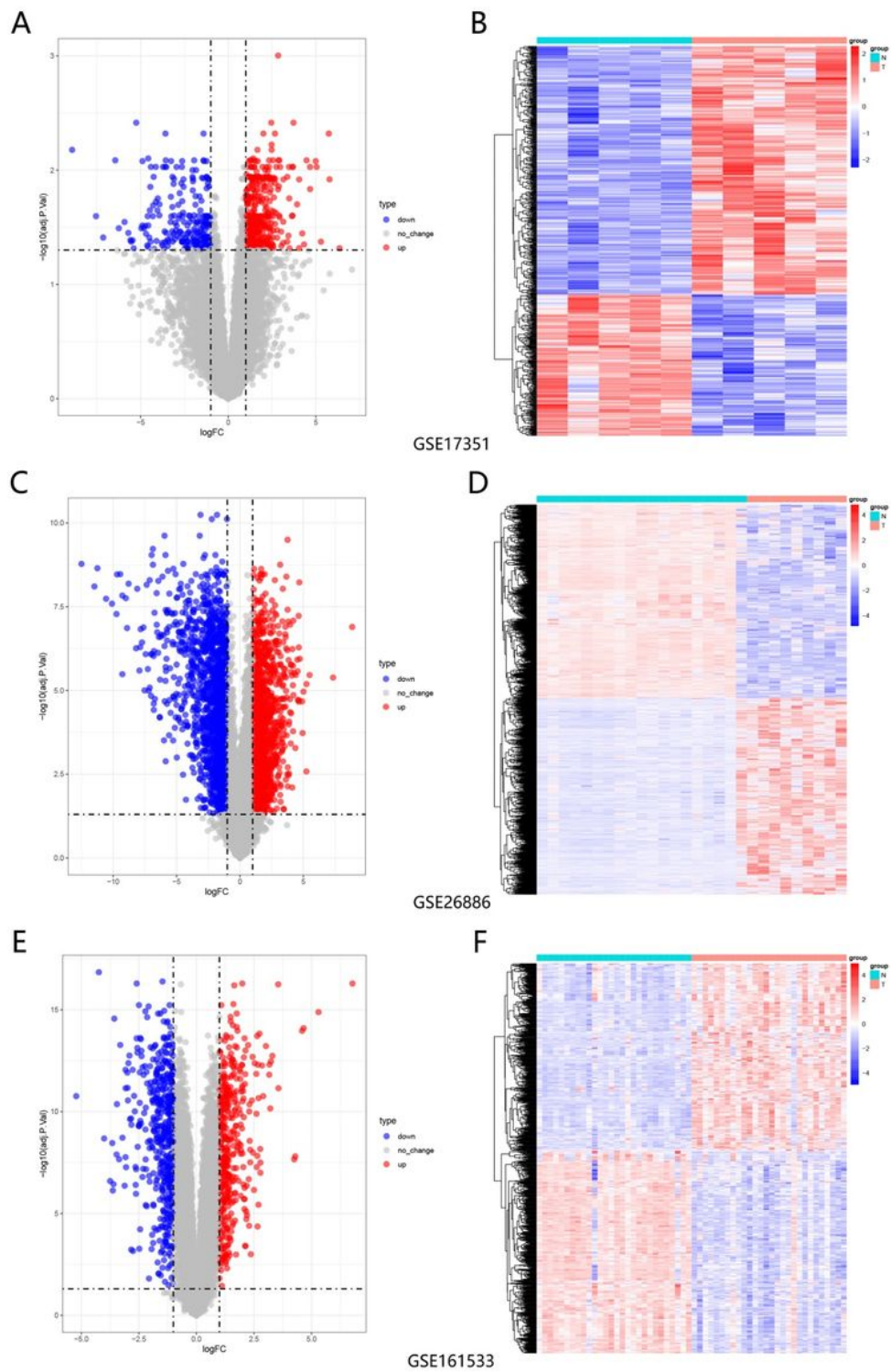


Figure 2

Volcano plot and heat map of DEGs of three gene chips. Red highlighted represents higher expression genes, while blue highlighted represents lower expression genes.

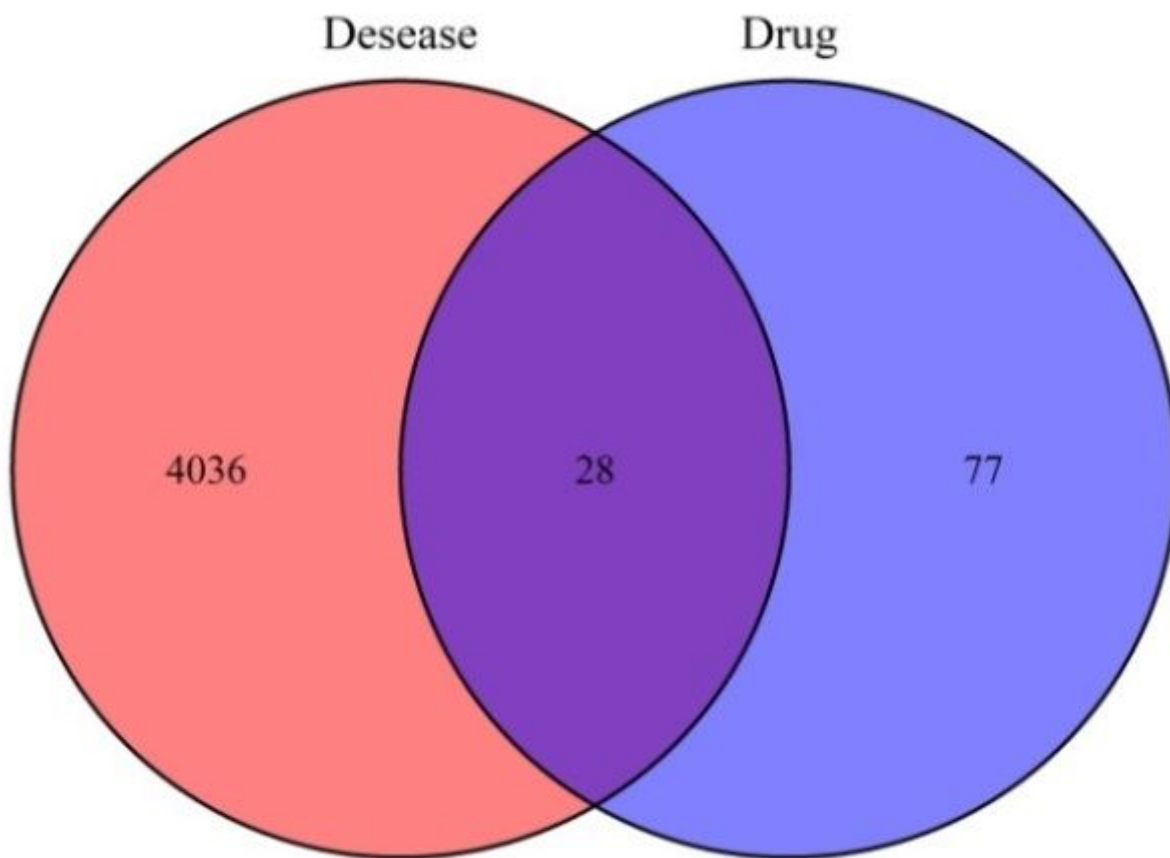


Figure 3

Venn diagram of drug targets and disease targets.

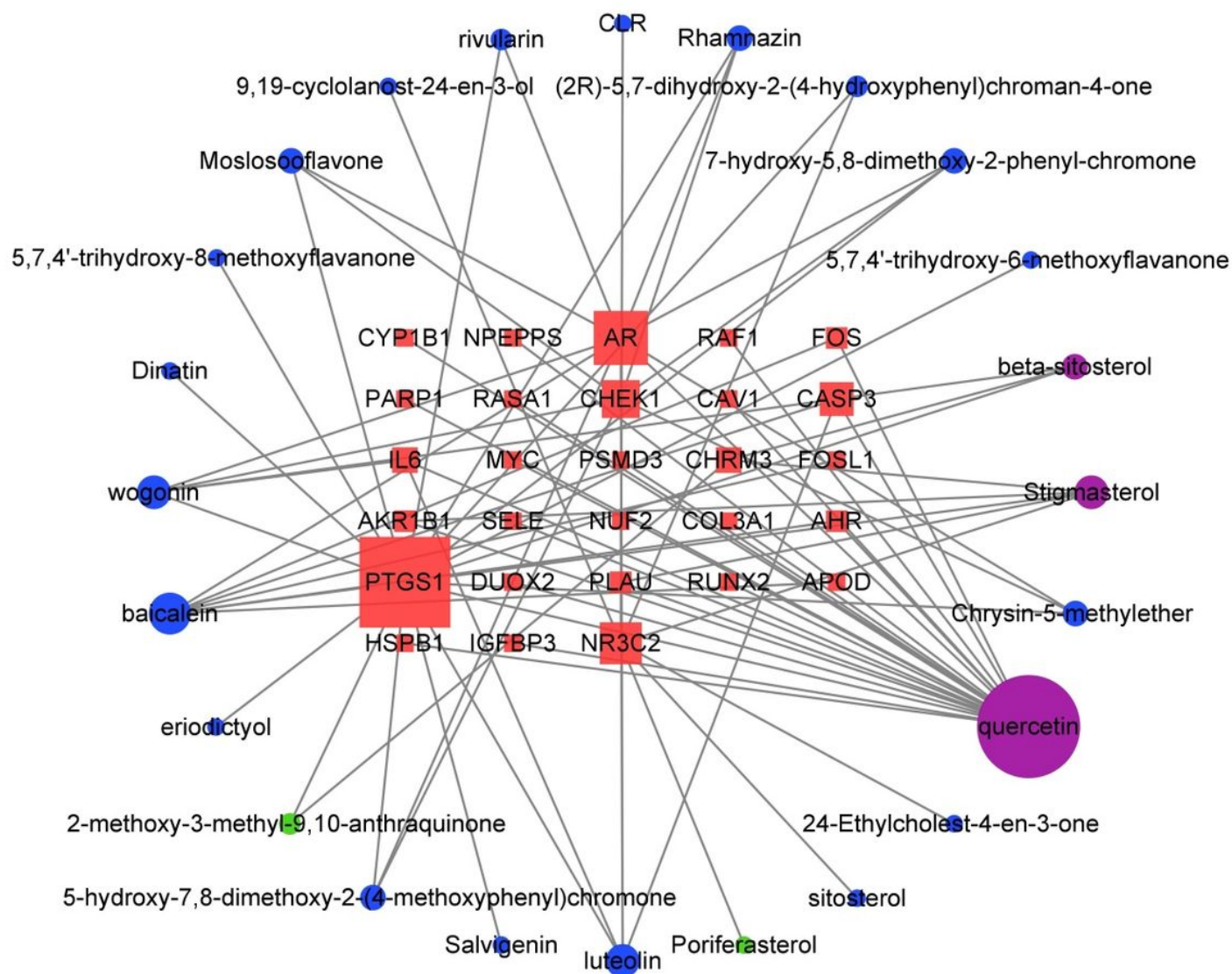
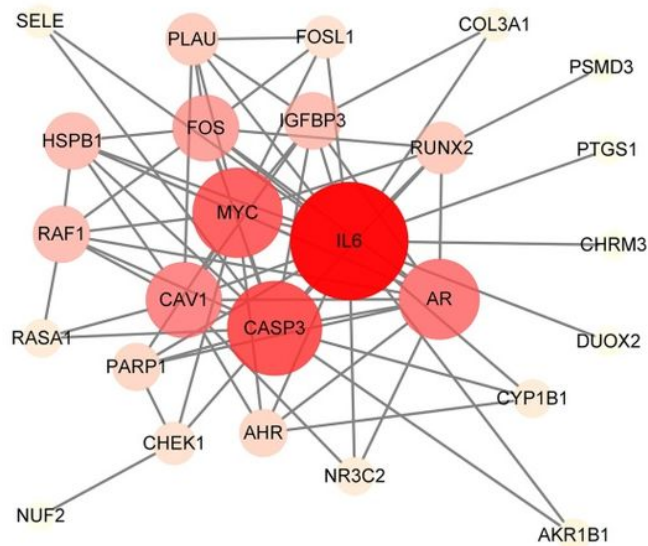


Figure 4

“Active compounds-targets” regulatory network. Red square nodes represent target genes, blue elliptic nodes represent active compounds in SB, green elliptic nodes represent active compounds in HD, and purple elliptic nodes represent active compounds shared by SB and HD.

A



B

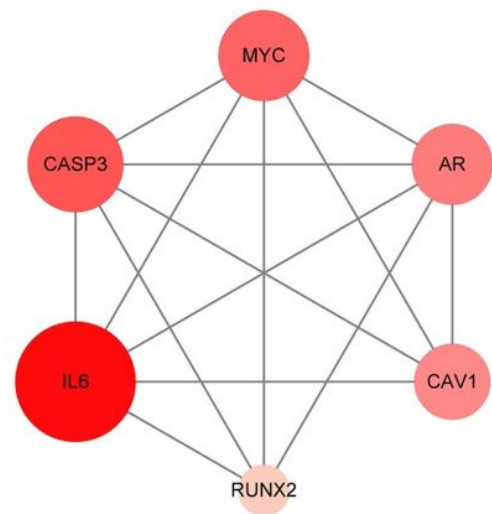


Figure 5

PPI network and core sub network for conceivable therapeutic targets. (A) The PPI network of 28 potential therapeutic targets; (B) The PPI core sub network of 6 hub genes; The node degree values are shown as tiny to big circles and yellow to red hues.

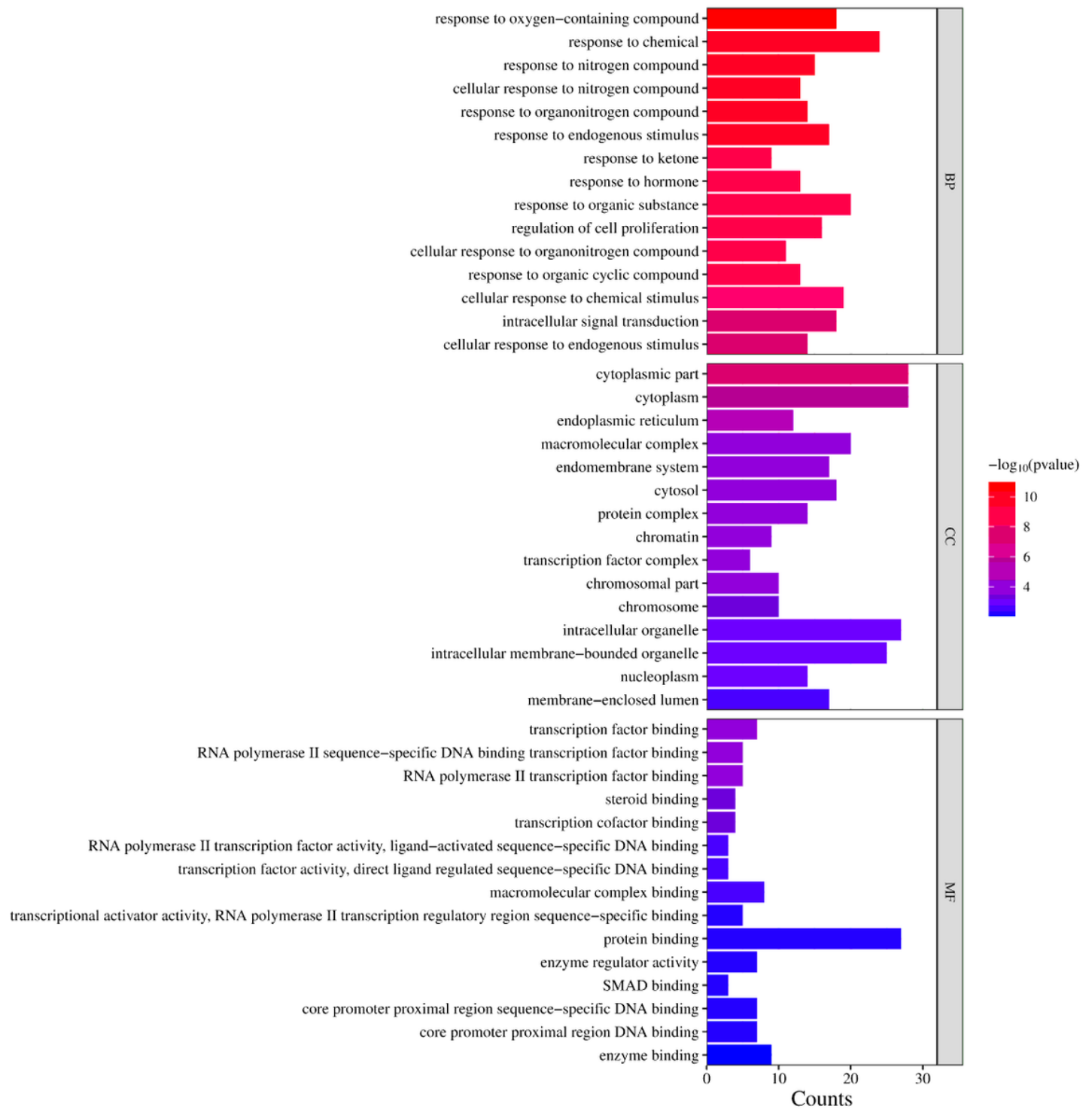


Figure 6

GO enrichment analysis.

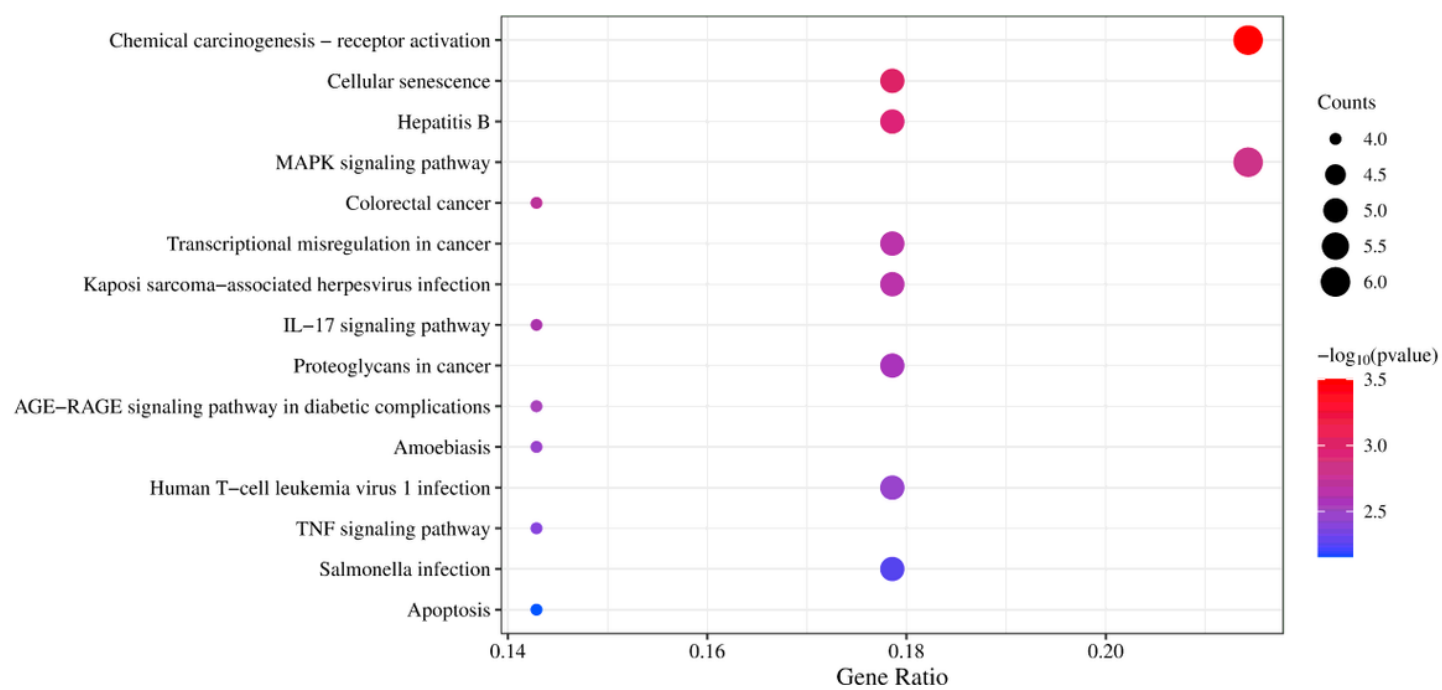


Figure 7

KEGG enrichment analysis.

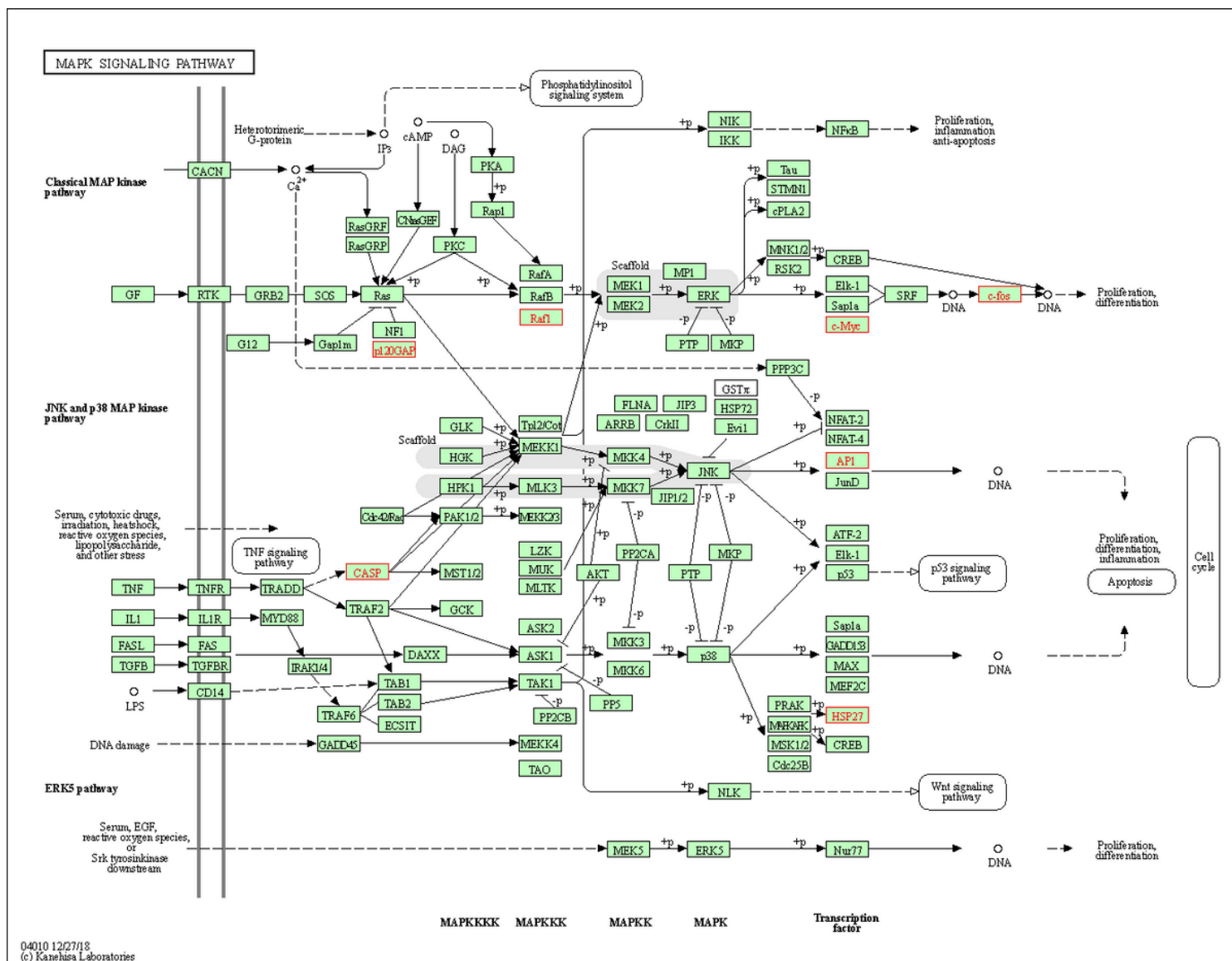


Figure 8

MAPK signaling pathway enrichment diagram. The red highlighted represent the target protein nodes related to this research.

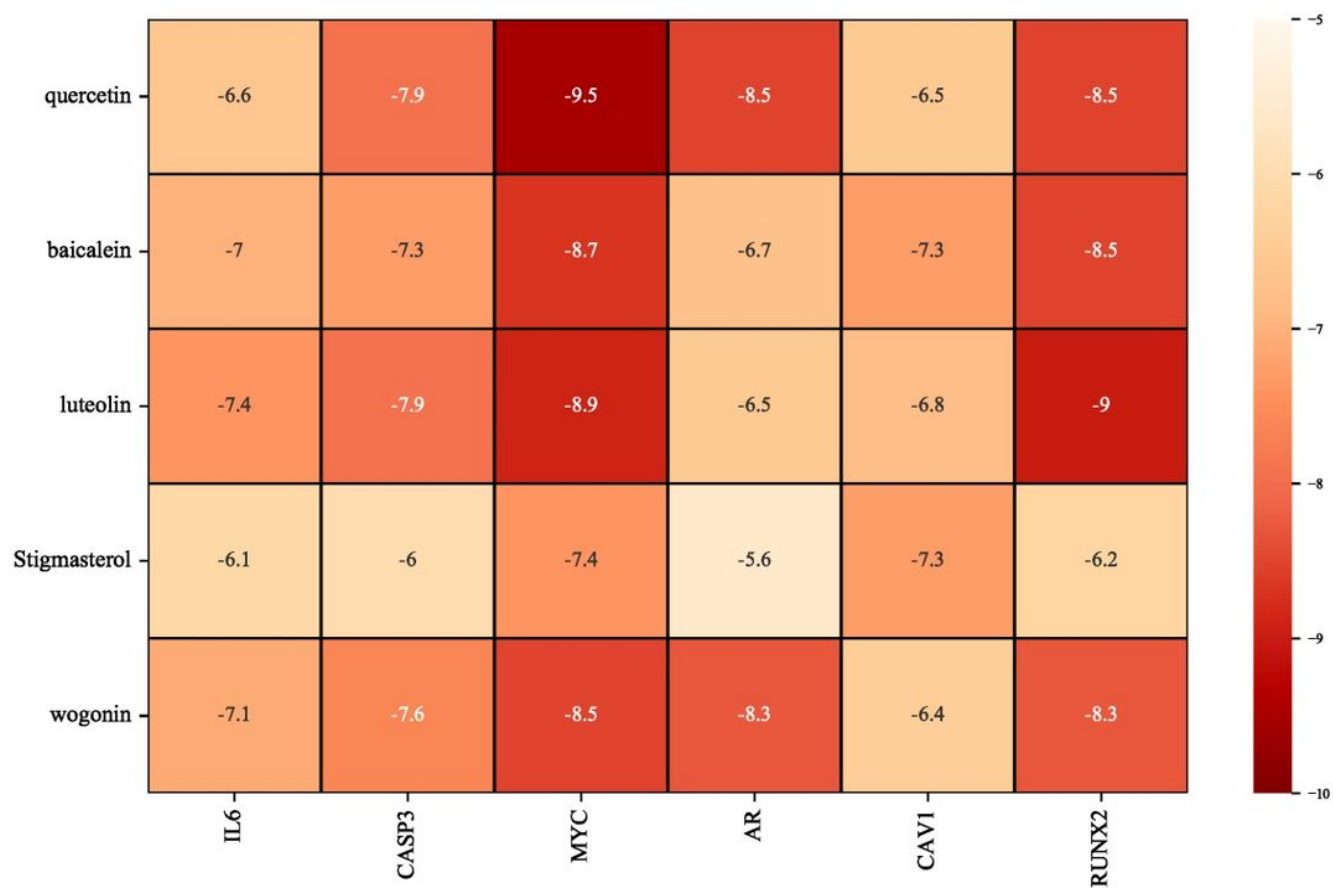


Figure 9

Matrix heat map of the molecular docking affinity scores (kcal/mol).

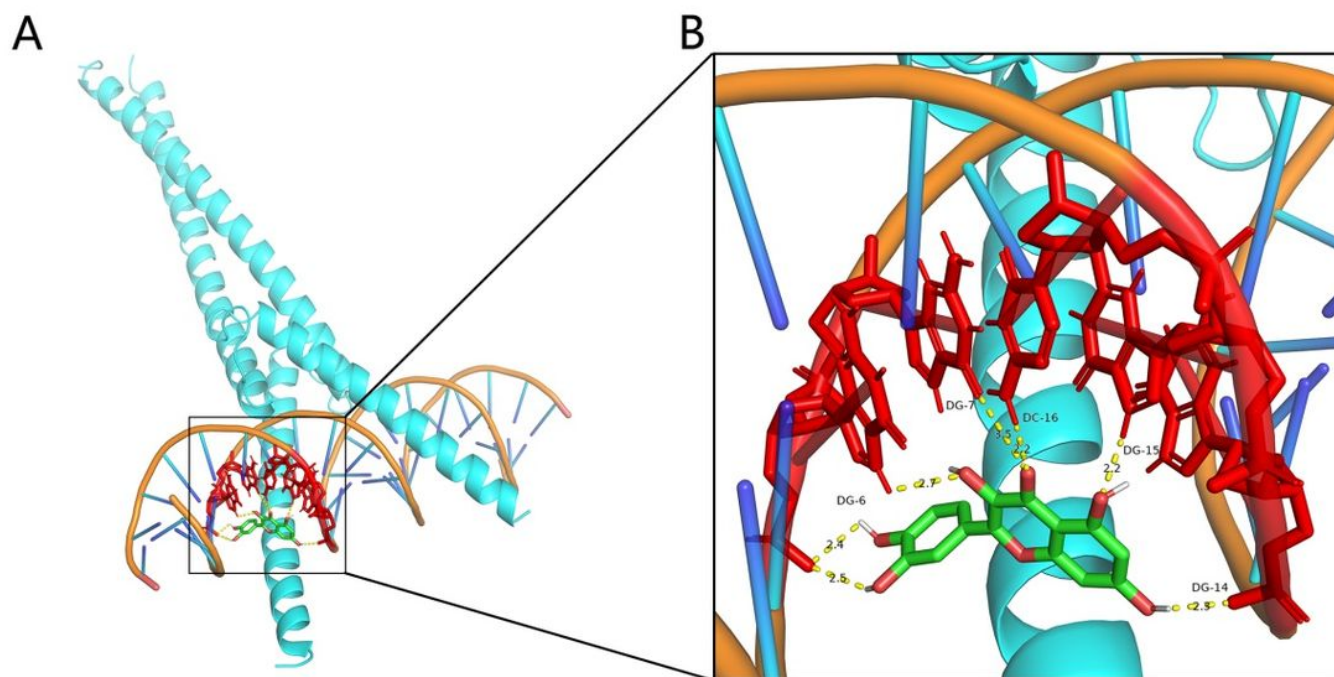


Figure 10

3D diagram of molecular docking between MYC and quercetin.