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Exploring the Mechanism of Action of the "Scutellaria Barbata D.Don-Prunella Vulgaris L." Herb Pair in the Treatment of Lung Cancer Using Network Pharmacology and Molecular Docking Techniques

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Abstract: ***Objective: To explore the mechanism of action of the herbal pair "Scutellaria barbata D.Don-Prunella vulgaris L." in the treatment of lung cancer through network pharmacology and molecular docking techniques. Methods: Active ingredients and their targets of Scutellaria barbata D.Don and Prunella vulgaris L. were collected and screened from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Disease-related targets were obtained and screened from the Genecards and OMIM databases. The intersection Venn diagram of the targets of "Scutellaria barbata D.Don-Prunella vulgaris L." and lung cancer disease targets was obtained using R 4.4.1 software and packages such as "ggvenn". A drug-active ingredient-target-disease association network was constructed in Cytoscape 3.10.0, and core active ingredients were screened using the Analyze Network function. A PPI network for drug-disease common targets was constructed using the String database website, and the TSV format of protein interaction relationship files was imported into Cytoscape 3.10.0 software,*** **install and run CytoHubba to calculate and obtain the core targets in the network.** ***GO function and KEGG pathway enrichment analyses were performed on drug-disease common targets using R 4.4.1 software. Finally, molecular docking validation was performed on core ingredients and core targets using AutoDock, and the three best binding molecular docking patterns were displayed using PyMol software. Results: A total of 33 active drug components and 108 drug-disease common targets were obtained. Among them, there are 5 core active components: quercetin, luteolin, wogonin, kaempferol, and baicalein; core targets include TP53, AKT1, JUN, HSP90AA1, etc. GO analysis yielded 2, 010 related entries. KEGG analysis identified 147 signaling pathways. Molecular docking showed that the core active components have strong affinity with the core targets. Conclusion: The drug pair of Scutellaria barbata D.Don and Prunella vulgaris L. may exert anti-tumor effects by acting on targets such as TP53, AKT1, JUN, HSP90AA1, and through signaling pathways like PI3K-AKT, inhibiting tumor cell proliferation, promoting tumor cell apoptosis, suppressing its proliferation, differentiation, and metastasis, thereby achieving therapeutic effects on lung cancer.***

Keywords: Scutellaria barbata D.Don, Prunella vulgaris L., Network pharmacology, Network pharmacology, AkT1**.**

1. Introduction

According to global cancer statistics, lung cancer has the highest incidence and mortality rates among all types of cancer, and this trend is expected to continue to rise in the future, indicating that lung cancer has become one of the most threatening cancers globally. As a high-incidence area for lung cancer, China's incidence and mortality rates for the disease are both more severe than the global average [1]. To address this public health challenge, measures must be taken to strengthen lung cancer prevention, promote healthy lifestyles, reduce tobacco harm, improve air quality, and screen high-risk populations. In the medical field, the treatment of lung cancer typically involves various methods such as surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy [2, 3]. Although these methods have their advantages, they still face challenges of recurrent or worsening conditions. The five-year survival rate is not ideal. Patients may also encounter complications during treatment, further increasing the physical and mental burden. In such cases, traditional Chinese medicine, as an important supplement to the comprehensive treatment plan for lung cancer, often plays a multifaceted role. Through its unique therapeutic effects, traditional Chinese medicine can significantly alleviate the side effects of radiotherapy and chemotherapy, thereby helping patients relieve the dual pressure of body and mind. At the same time, it controls the development of tumors, enhances the treatment effect, and reduces the possibility of recurrence. In addition, it can improve the physical function of patients during treatment and the rehabilitation period, enhance immunity, improve quality of life and survival rate, and is deeply trusted by patients [4].

Contemporary scholars of Traditional Chinese Medicine (TCM) generally believe that the causes of lung cancer are closely related to factors such as dampness, toxicity, phlegm, and blood stasis. The long-term cumulative effects of these factors are the main pathological mechanisms leading to lung cancer. Medicines with heat-clearing and detoxifying properties are often used in combination in the clinical treatment of lung cancer. Scutellaria barbata D.Don and Prunella vulgaris L., as commonly used herbs in the treatment of lung cancer in TCM, have shown significant clinical effects through syndrome differentiation and treatment [5]. Scutellaria barbata D.Don is a frequently used drug in the treatment of tumors, with its main functions including clearing heat and detoxifying, and promoting blood circulation and diuresis [6]. Prunella vulgaris L., a widely used and well-known herb in the field of TCM, can clear heat and reduce swelling, soften hardness and dissipate masses [7]. Recent studies have further revealed that Scutellaria barbata D.Don not only has an inhibitory effect on general lung cancer

cells but also on lung cancer stem cells [8]. Additionally, in cellular experiments, Prunella vulgaris L. was found to effectively increase the expression level of the pro-apoptotic protein caspase-3 in lung cancer cells A549, significantly increasing the content of caspase-3 protein within A549 cells. Through this core mechanism, it exerts an inhibitory effect, guiding lung cancer cells into the apoptotic process, thereby effectively limiting their ability to over-proliferate [9].

The traditional Chinese medicines Scutellaria barbata D.Don and Prunella vulgaris L. both contain multiple bioactive compounds, but the specific mechanisms when used in combination are not yet clear. The first step of this study employed network pharmacology methods to explore the multi-target action information of multiple effective active ingredients present in these two medicinal materials. Furthermore, molecular docking technology was used to simulate and verify the results obtained from network pharmacology, screening out those with higher reliability. The combination of these two methods can efficiently and comprehensively analyze the potential mechanisms of the “Scutellaria barbata D.Don-Prunella vulgaris L.” pair in lung cancer treatment. This research provides a theoretical basis and new insights for the application of traditional Chinese medicine in the treatment of lung cancer, and also offers guidance and reference for subsequent drug development and experimental verification work.

2. Methodology

2.1 Screening "Scutellaria Barbata D.Don-Prunella Vulgaris L." Drug Pair Active Ingredients and Standardized Naming of Drug Targets

By accessing the online database at http://tcmspw.com/tcmsp.php, searches were conducted for Scutellaria barbata D.Don and Prunella vulgaris L., two traditional Chinese medicines. On their active ingredients page, components with high bioavailability and drug potential were filtered out, setting specific screening criteria: oral bioavailability (OB)≥30%, and drug likeness (DL) ≥ 0.18. With these criteria, active ingredients that are easily absorbed by the human body and exhibit high drug potential in their chemical structure were identified. Their Related Targets, which are the information on target proteins associated with these components, were obtained. These target proteins are key to drug action. Finally, the standardized gene names of these drug targets were queried in the Uniprot database (https: //www.uniprot.org/).

2.2 Obtain Lung Cancer Disease Targets and Draw a Venn Diagram of Disease-drug Intersection Targets

Search with the keyword "lung cancer" in the GeneCards database (https://www.genecards.org/) and the OMIM database (https://omim.org/), and obtain scientifically validated lung cancer-related gene targets. Download these data into Excel, and use the median filtering method in the relevance score column. Filter out low-score targets without research value in the Genecards database. After calculation, the optimal median is determined to be 26.85918045, and then filter by setting the Relevance score filtering value to ≥26.85918045. Summarize the targets obtained from the two databases in Excel, remove duplicates, and obtain the final lung cancer disease targets. Using R 4.4.1 software, install and run packages such as "ggvenn", input the files of the drug targets of the "Scutellaria barbata D.Don - Prunella vulgaris L." drug pair and lung cancer disease targets, export a Venn diagram showing the intersection of both, and obtain the drug-disease common targets.

2.3 Constructing a Drug-Active Ingredient-Common Target-Disease Network

To deeply explore the complex interactions among drugs, active ingredients, drug-disease common targets, and diseases, we have imported various relevant data and information into Cytoscape 3.10.0 software. By constructing a comprehensive network model, we demonstrate the interrelationships between drugs, active ingredients, common targets, and diseases. Subsequently, we opened Cytoscape 3.10.0 software and found the Analyze Network program in the Tools menu to perform network topology analysis, in order to better understand the structure and characteristics of this network model. The Analyze Network program enables the identification of key nodes and connections in the network, thereby filtering out drug active ingredients that may play a core role in the treatment of lung cancer.

2.4 Target Network Construction and Key Target Selection

Input the common targets of drugs and diseases into the String platform for PPI analysis, thereby generating a PPI network map and a TSV format file of protein interaction relationships. Then, import the TSV file containing the protein interaction information into the Cytoscape 3.10.0 software for further visual analysis. Install and run CytoHubba in the app store of Cytoscape 3.10.0 software, and after calculation, obtain the top 10 key targets with the highest degree values in the PPI network and their corresponding degree value data. In the PPI network, the larger the degree value of a node, the more important role it usually plays in the overall network structure.

2.5 GO and KEGG Enrichment Analysis

Open R 4.1.1 software and perform in-depth mining of gene data's intrinsic value for the common target gene file using packages such as "DOSE", "org.Hs.eg.db", "pathview", and "clusterProfiler". These packages allow for the execution of complex analyses to uncover significant biological information from gene data. Conduct GO analysis, which categorizes gene functions into three major groups: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). This helps to understand which genes play important roles in biological processes, which genes have specific localizations in cellular components, and which genes exhibit significant characteristics in molecular functions under specific conditions. Perform KEGG pathway analysis to understand the roles of genes in biological pathways, especially in cell signaling and metabolism. Then, visualize the results. To ensure the reliability of the analysis results, set the filtering condition to a p-value less than 0.01.

2.6 Molecular Docking Verification

Conduct molecular docking validation with the five key active ingredients and the top four core targets ranked by degree value, namely TP53, AKT1, JUN, and HSP90AA1, to ensure that their binding is stable and biologically significant. The 3D structures of the four core targets and the SDF format files of the five core ingredients will be downloaded from the PDB database (http: //www.rcsb.org/) and the PubChem database, respectively. The core targets will be prepared for docking by removing water and the original ligands using PyMol software, and the SDF format files of the core ingredients will be converted to mol2 format using Chem3D software for subsequent molecular docking experiments. Open AutoDock 1.5.7 software to convert the core targets and core ingredients into pdbqt format, then perform molecular docking validation to simulate the molecular-level interaction between the two, obtaining the binding energy value as a key indicator to evaluate their binding affinity. When the binding energy value is less than -5.0 kcal/mol, it indicates that the ligand and receptor have good binding force and have the potential to maintain a long-lasting binding state in the biological body. A value less than -8.0 kcal/mol indicates a strong binding force between the two and may increase the ligand's selectivity for a specific receptor. Finally, use PyMol software to visualize the top three results based on binding energy data.

3. Results

3.1 Screening Results of Drug Active Ingredients and Targets

After threshold screening, a total of 36 effective ingredients were extracted from Scutellaria barbata D.Don and Prunella vulgaris L.. Specifically, Scutellaria barbata D.Don provided 29 effective ingredients, while Prunella vulgaris L. contributed 11 effective ingredients, among which 4 ingredients were common to both drugs. These overlapping ingredients include quercetin, luteolin, beta-sitosterol, and stigmasterol. The active ingredients of the drugs, along with their Mol ID, OB, and DL data, were compiled into a table (see Table 1). After excluding duplicates, the two drugs had a total of 230 effective targets, which represent the sites of potential therapeutic effects of the drugs, converting them into corresponding gene names.

Table 1: Scutellaria barbata D.Don - Prunella vulgaris L. active ingredients

Traditional Chinese medicine	Mol ID	Molecule Name	OB (%)	DL
Prunella vulgaris L.	MOL000358	beta-sitosterol	36.91	0.75
Prunella vulgaris L.	MOL000422	kaempferol	41.88	0.24
Prunella vulgaris L.	MOL004355	Spinasterol	42.98	0.76
Prunella vulgaris L.	MOL000449	Stigmasterol	43.83	0.76
Prunella vulgaris L.	MOL004798	delphinidin	40.63	0.28
Prunella vulgaris L.	MOL000006	luteolin	36.16	0.25
Prunella vulgaris L.	MOL006767	Vulgaxanthin-I	56.14	0.26
Prunella vulgaris L.	MOL006772	poriferasterol monoglucoside_qt	43.83	0.76
Prunella vulgaris L.	MOL006774	stigmast-7-enol	37.42	0.75
Prunella vulgaris L.	MOL000737	morin	46.23	0.27
Prunella vulgaris L.	MOL000098	quercetin	46.43	0.28
Scutellaria barbata D.Don	MOL001040	(2R)-5, 7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	42.36	0.21
Scutellaria barbata D.Don	MOL012245	5, 7, 4'-trihydroxy-6-methoxyflavanone	36.63	0.27
Scutellaria barbata D.Don	MOL012246	5, 7, 4'-trihydroxy-8-methoxyflavanone	74.24	0.26
Scutellaria barbata D.Don	MOL012248	5-hydroxy-7, 8-dimethoxy-2-(4-methoxyphenyl) chromone	65.82	0.33
Scutellaria barbata D.Don	MOL012250	7-hydroxy-5, 8-dimethoxy-2-phenyl-chromone	43.72	0.25
Scutellaria barbata D.Don	MOL012251	Chrysin-5-methylether	37.27	0.2
Scutellaria barbata D.Don	MOL012252	9, 19-cyclolanost-24-en-3-ol	38.69	0.78
Scutellaria barbata D.Don	MOL002776	Baicalin	40.12	0.75
Scutellaria barbata D.Don	MOL012254	campesterol	37.58	0.71
Scutellaria barbata D.Don	MOL000953	CLR	37.87	0.68
Scutellaria barbata D.Don	MOL000358	beta-sitosterol	36.91	0.75
Scutellaria barbata D.Don	MOL012266	rivularin	37.94	0.37
Scutellaria barbata D.Don	MOL001973	Sitosteryl acetate	40.39	0.85
Scutellaria barbata D.Don	MOL012269	Stigmasta-5, 22-dien-3-ol-acetate	46.44	0.86
Scutellaria barbata D.Don	MOL012270	Stigmastan-3, 5, 22-triene	45.03	0.71
Scutellaria barbata D.Don	MOL000449	Stigmasterol	43.83	0.76
Scutellaria barbata D.Don	MOL000173	wogonin	30.68	0.23
Scutellaria barbata D.Don	MOL001735	Dinatin	30.97	0.27
Scutellaria barbata D.Don	MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76
Scutellaria barbata D.Don	MOL002714	baicalein	33.52	0.21
Scutellaria barbata D.Don	MOL002719	6-Hydroxynaringenin	33.23	0.24
Scutellaria barbata D.Don	MOL002915	Salvigenin	49.07	0.33
Scutellaria barbata D.Don	MOL000351	Rhamnazin	47.14	0.34
Scutellaria barbata D.Don	MOL000359	sitosterol	36.91	0.75
Scutellaria barbata D.Don	MOL005190	eriodictyol	71.79	0.24
Scutellaria barbata D.Don	MOL005869	daucostero_qt	36.91	0.75
Scutellaria barbata D.Don	MOL000006	luteolin	36.16	0.25
Scutellaria barbata D.Don	MOL008206	Moslosooflavone	44.09	0.25
Scutellaria barbata D.Don	MOL000098	quercetin	46.43	0.28

3.2 Obtaining Venn Diagram of Lung Cancer Disease Targets and Disease-Drug Intersection Targets

A total of 26, 719 lung cancer targets were obtained from the GeneCards database. After median value filtering, the disease targets from both the GeneCards and OMIM databases were combined and duplicates were removed, resulting in a total of 887 disease targets. Using the "ggvenn" package and other programs in R 4.4.1 software, the intersection of these 887 disease targets and 230 drug action targets was taken,

resulting in 108 intersection targets presented in a Venn diagram format (see Figure 1). These targets may be potential targets for the treatment of lung cancer with Scutellaria barbata D.Don and Prunella vulgaris L..

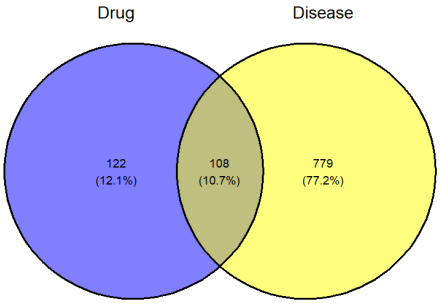

Figure 1: Drug-Disease Target Venn Diagram

3.3 Construction of Drug-Active Ingredient-Common Target-Disease Network

By fully utilizing the bioinformatics software Cytoscape, a detailed drug-active ingredient-target-disease association network map has been constructed.(see Figure 2) In the map, nodes of different shapes represent different elements: triangles represent traditional Chinese medicine entities, ellipses represent active ingredients in individual drugs, diamonds represent shared active ingredients between two drugs, while rectangles and hexagons represent potential targets and disease nodes respectively. Drugs and their corresponding unique active ingredients are given the same color scheme. Through topological data analysis of the network map, some key high-connectivity nodes have been revealed, such as quercetin (degree value of 89), luteolin (degree value of 44), wogonin (degree value of 31), kaempferol (degree value of 29), and baicalein (degree value of 21). The high degree values of these nodes directly reflect their frequency of interaction and influence within the network. These active ingredients not only have direct interactions with multiple targets, indicating their multifunctionality in drug action, but they may also have synergistic effects with other active ingredients, thereby enhancing the overall therapeutic efficacy of the drugs. This finding strongly supports the therapeutic strategy of Scutellaria barbata D.Don and Prunella vulgaris L. regulating multiple active ingredients through multidimensional approaches and acting on multiple targets to achieve their anti-tumor effects. These core ingredients play a crucial role in the treatment process, and they may become key active molecules in overcoming lung cancer, providing new insights and directions for future drug development.

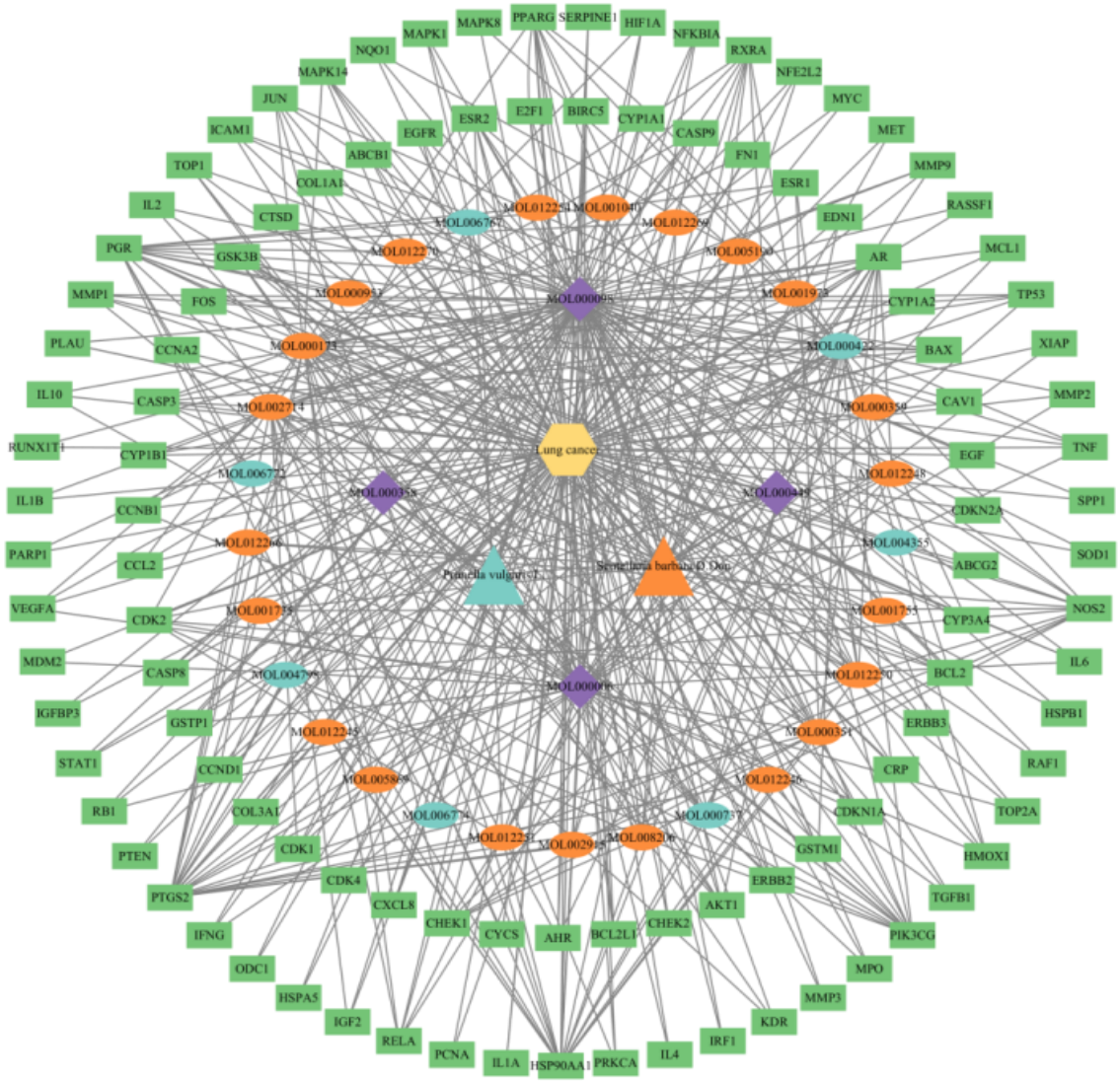

Figure 2: Drug-Active Ingredient-Common Target-Disease Network

3.4 Target Network Construction and Core Target Selection

In the String database, we obtained the PPI network graph and the protein interaction relationship files in TSV format. Unrelated targets in the PPI network graph were hidden (see Figure 3). Subsequently, we exported the protein interaction relationship data in TSV format and visualized it using the Cytoscape 3.10.0 software (see Figure 4). The network graph contains 105 nodes and 463 edges, where the targets are presented with circular icons, and the color scheme uses orange. The node sizes are arranged according to their degree values in descending order, and the color shade changes with the increase or decrease of node degree values, to visually display the connection strength between nodes. Using the CytoHubba plugin for analysis, the top 10 targets ranked by degree value are TP53 (degree value of 47), AKT1 (degree value of 28), JUN (degree value of 25), HSP90AA1 (degree value of 23), TNF (degree value of 22), IL6 (degree value of 22), ESR1 (degree value of 22), BCL2 (degree value of 21), CCND1 (degree value of 20), and MYC (degree value of 18), respectively. In this network structure, nodes with high value often reside in core positions, indicating that they may play a crucial role in the pathogenesis of lung cancer. These nodes may have a profound impact on the occurrence and progression of lung cancer, involving numerous signaling pathways and biological processes. As potential drug targets, they are of high value, and inhibiting these key nodes could have a significant therapeutic effect on the disease. In the study of the mechanism of combined treatment of lung cancer with Scutellaria barbata D.Don and Prunella vulgaris L., the study of these core targets is particularly crucial.

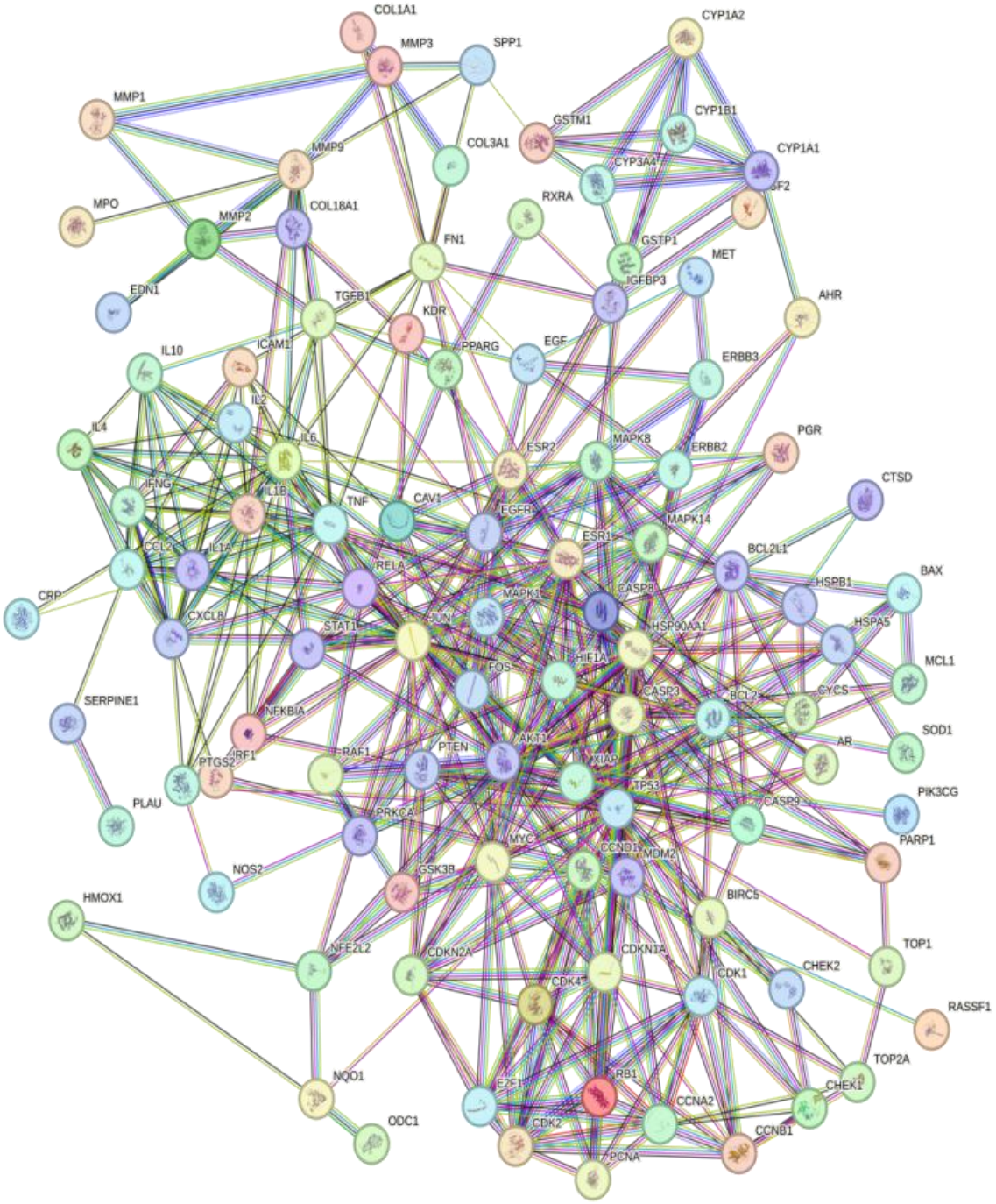

Figure 3: PPI network graph

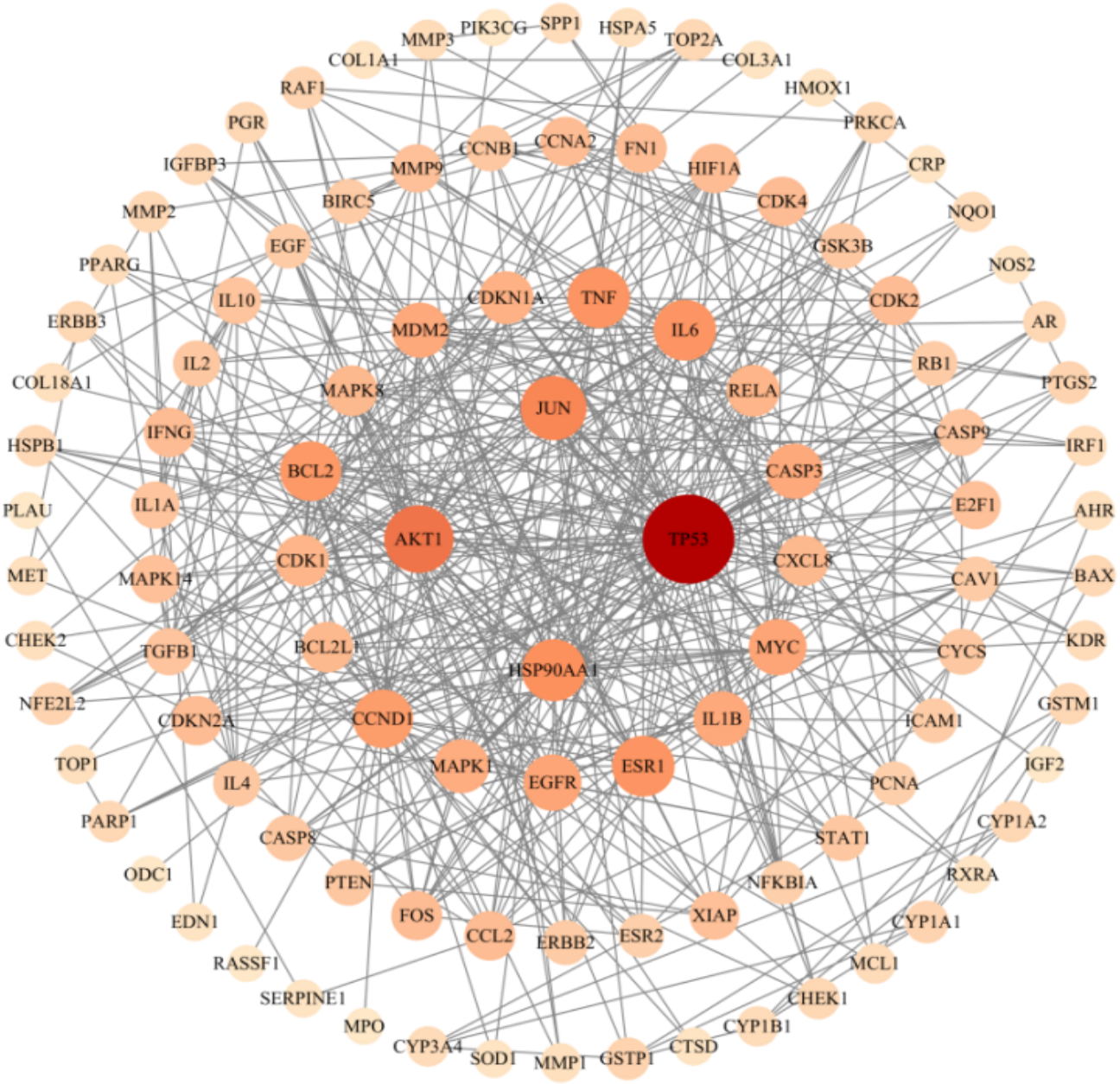

Figure 4: PPI network graph visualization

3.5 Functional and Pathway Analysis Results

Through enrichment analysis, a total of 2, 010 GO entries that showed significant differences at a significance level of $P < 0.01$ were filtered out. The specific distribution of these entries in the three major categories is as follows: The biological process category dominates with 1, 847 entries, the cellular component category includes 46 entries, and the molecular function category contains 117 entries. The top 10 entries with the highest enrichment in each category were selected and presented in chart form for further analysis and research (see Figure 5). In terms of biological processes, these entries mainly focus on key biological events such as response to reactive oxygen species, response to lipopolysaccharide, regulation of apoptotic signaling pathway, and negative regulation of apoptotic signaling pathway. The entries in the cellular component category mainly involve key components of cellular structure and function, including cyclin−dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, protein kinase complex, and Bcl-2 family protein complex. As for molecular functions, these entries cover interactions and functions between biological molecules, such as ubiquitin-like protein ligase binding, DNA-binding transcription factor binding, kinase regulatory activity, and protein kinase regulatory activity. In addition, KEGG pathway enrichment analysis revealed 147 metabolic and signal transduction pathways that were significantly different at a significance level of $P < 0.01$. The top 20 pathways with the highest enrichment were selected and visually displayed using bubble charts (see Figure 6).These pathways involve multiple important fields, including the PI3K-Akt signaling pathway, lipid and atherosclerosis, proteoglycans in cancer, small cell lung cancer, p53 signaling pathway, and non-small cell lung cancer.

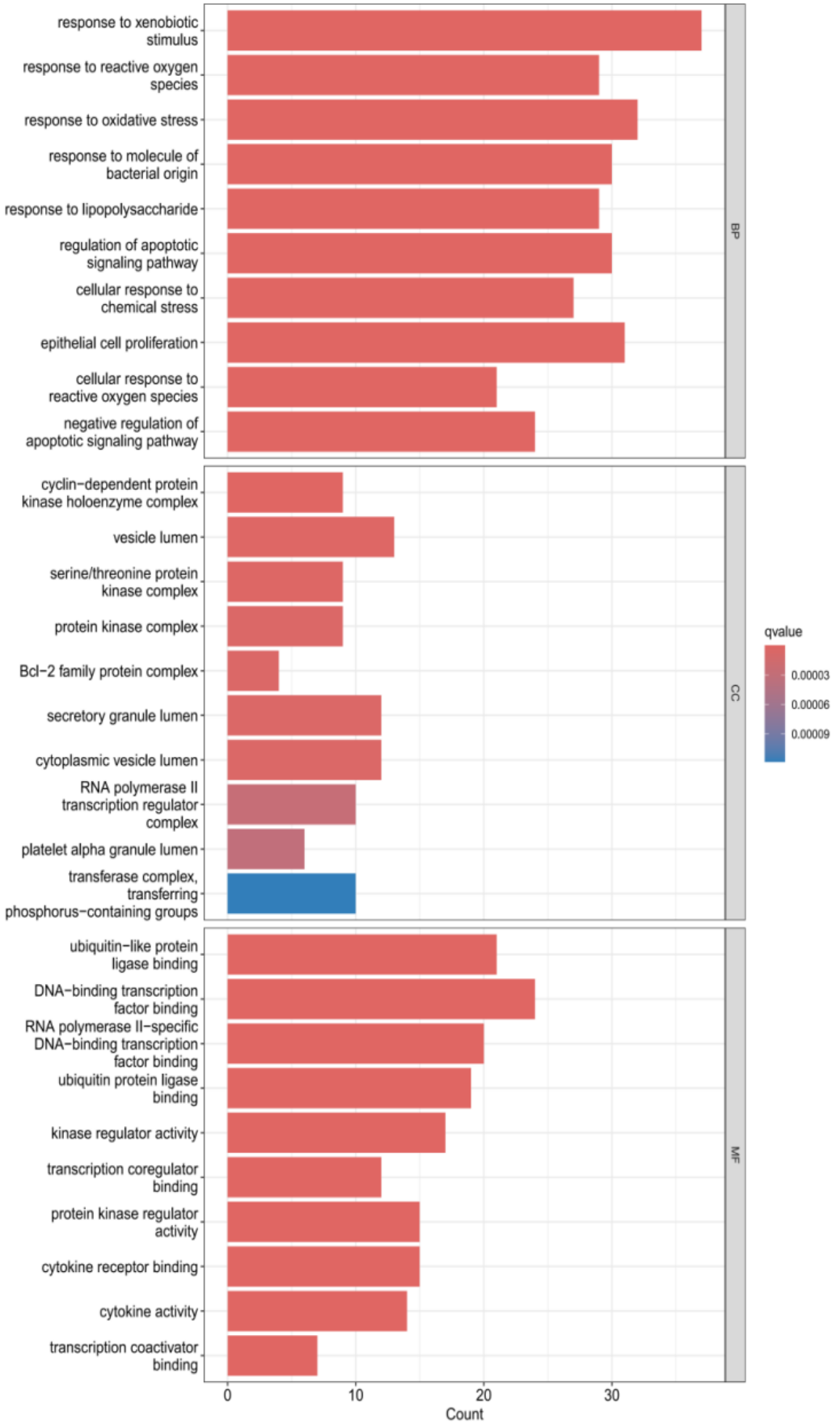

Figure 5: GO Functional Enrichment Analysis

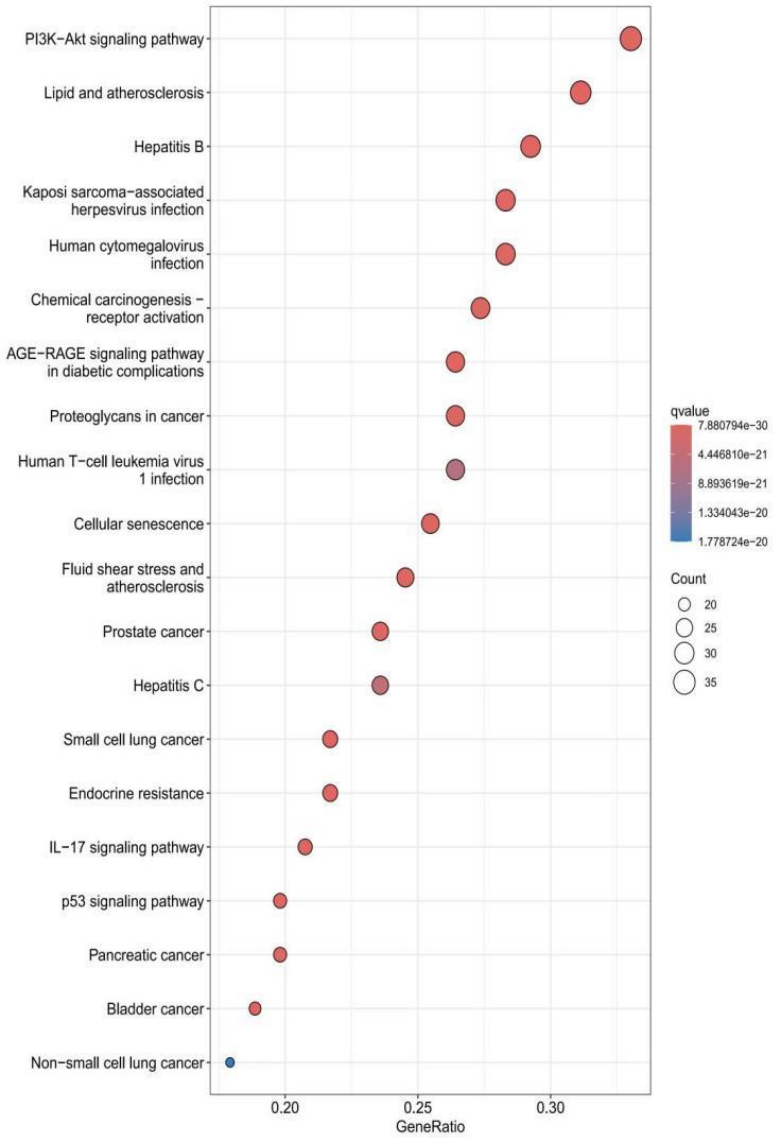

Figure 6: KEGG pathway enrichment analysis

3.6 Molecular Docking Results

This study selected five key components and conducted molecular docking experiments with the top four targets in the PPI network, TP53, AKT1, JUN, and HSP90AA1. The experimental results indicated that the effective binding ability of all tested compounds with specific receptor targets was clearly validated. The data showed that the binding energy values between these ligands and proteins were all below -5 kcal/mol (see Table 2). Ligands and receptors have the potential to maintain a binding state for a relatively long time within the body, which provides an important theoretical basis for subsequent research and the development of anti-lung cancer drugs based on Scutellaria barbata D.Don and Prunella vulgaris L.. In addition, the study revealed that some ligands exhibited strong binding activity to multiple proteins. To visually display these results, the previous degree value data was integrated, and the top three molecular docking patterns with the strongest binding force were drawn in the PyMol software (see Figure 7). Among them, the binding energy data of quercetin with AKT1 performed the best, with a binding energy of -8.3 kcal/mol, indicating a strong binding force between the two. Following that, the binding energy of baicalein with AKT1 reached -7.98 kcal/mol, and the binding energy of kaempferol with HSP90AA1 reached -7.67 kcal/mol. These pattern diagrams not only provide a visual display but also help to deeply understand the interaction mechanism between ligands and proteins, clearly showing the hydrogen bonds formed between the ligands and specific amino acids of the receptor.

Table 2: Binding energy of key active ingredients with some core targets(kcal/mol)

Active ingredient	TP53	AKT1	JUN	HSP90AA1
quercetin	-6.58	-8.3	-5.29	-7.41
luteolin	-6.9	-7.46	-5.79	-7.58
wogonin	-6.79	-7.15	-5.14	-7.11
kaempferol	-5.8	-7.38	-6.3	-7.67
baicalein	-6.61	-7.98	-5.79	-7.02

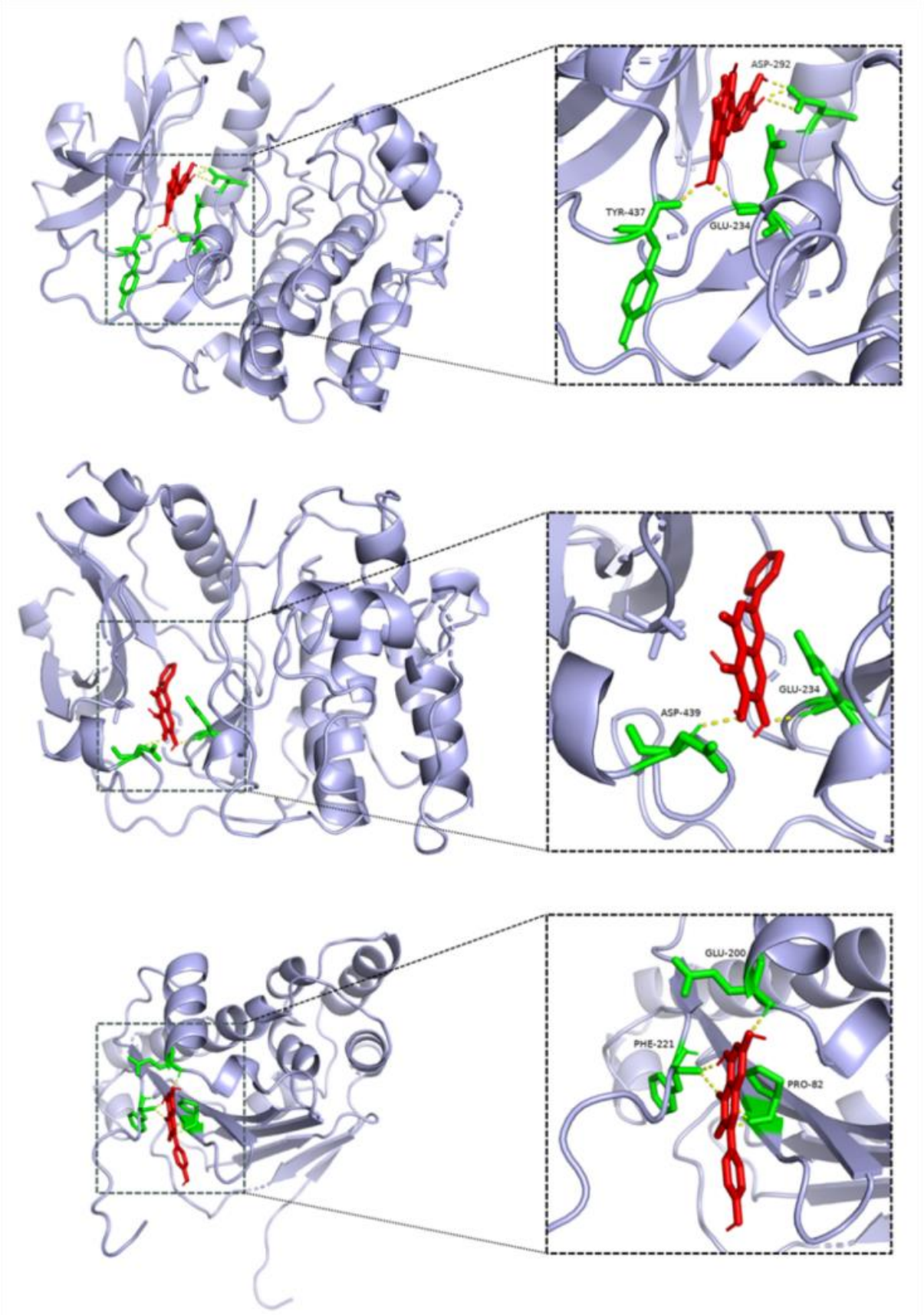

Figure 7: Molecular docking patterns of quercetin with AKT1, baicalein with AKT1, and kaempferol with HSP90AA1

4. Discussion

In the theoretical system of Traditional Chinese Medicine (TCM), the formation of lung cancer is considered to be related to the accumulation of internal heat toxins. These heat toxins may originate from external pathogenic influences or be caused by internal factors such as emotional imbalance, improper diet, etc., leading to obstructed circulation of qi and blood, and subsequently forming tumors. Moreover, the formation of lung cancer is closely related to

phlegm-dampness and blood stasis; the presence of heat toxins can promote the formation of phlegm-dampness and blood stasis, thereby exacerbating the progression of the disease. Some patients with lung cancer exhibit a deficiency of Zheng Qi (vital energy), leading to a weakened immune system, which provides an opportunity for the invasion of heat toxins. In the treatment of various patterns of lung cancer in TCM, the use of heat-clearing drugs is commonly combined. This is consistent with modern pharmacological research, as the heat-clearing and detoxifying components of Chinese herbs have been experimentally proven to inhibit the growth of tumor cells and induce their apoptosis.Chinese herbs with heat-clearing, blood-cooling, and detoxifying effects can clear internal heat toxins and cancer toxins to alleviate and treat the inflammation and symptoms caused by tumors. Scutellaria barbata D.Don and Prunella vulgaris L. are commonly used pairs of herbs for clearing heat and detoxifying in the treatment of lung cancer. These two herbs are applied in the treatment of various cancers and have provided ideal therapeutic effects in anti-tumor treatments. Their different active ingredients can act on multiple key targets, and it may be through this mechanism that they exert a synergistic effect in the treatment of lung cancer.

Five core active components were screened out from the "Scutellaria barbata D.Don-Prunella vulgaris L." herbal pair using network pharmacology methods. Studies have shown that quercetin can act as a SIRT1 activator, promoting apoptosis-induced autophagy in human lung cancer cells A549 and H1299 through the SIRT1/AMPK signaling pathway [10]. Another study found that quercetin inhibits the phosphorylation of the PI3K/AKT signaling pathway by binding to SIRT5 protein, thereby blocking DNA repair mechanisms in non-small cell lung cancer, leading to the accumulation of cell mutations and death [11]. Luteolin has been confirmed to treat lung cancer by inhibiting the EGFR-PI3K-AKT and integrin β1-FAK/Src signaling pathways, activating the MEK/ERK signaling pathway, and regulating the expression of migration-related factors such as AR, AIM2, and CCL2, to actively suppress tumor EMT and migration [12]. Research has found that Wogonin can inhibit the proliferation, migration, and invasion of lung cancer A549 and H460 cells by regulating the MMP1 and PI3K/AKT signaling pathways, and induce cell apoptosis [13]. Kaempferol can promote NSCLC cell autophagy by inhibiting Met and its downstream PI3K/AKT/mTOR signaling pathway, thereby inducing NSCLC cell apoptosis [14]. The method by which baicalein, contained in Scutellaria barbata D.Don, promotes apoptosis in lung cancer cells is achieved by downregulating the glutamine-mTOR metabolic pathway [15].

Through the analysis of the PPI network of shared targets between Scutellaria barbata D.Don and Prunella vulgaris L. in lung cancer treatment, it is known that the potential core targets of "Scutellaria barbata D.Don-Prunella vulgaris L." in treating lung cancer include TP53, AKT1, JUN, and HSP90AA1. The TP53 gene is often referred to as a tumor suppressor gene, but it frequently undergoes mutations in cancer. Approximately 50% of lung cancer cases have mutations in the TP53 gene, which accelerate tumor development by promoting the proliferation of abnormal cells. For example, in non-small cell lung cancer (NSCLC), TP53 mutations activate intracellular signaling pathways associated with EGFR translocation and exacerbate oncogenic characteristics driven by kras [16]. AKT1 is a member of the AKT kinase family, and AKT/protein kinase B (PKB) plays a central role in cell signaling pathways, responsible for regulating cell survival and death. Carcinogenic stimuli and growth factors can act together on AKT kinase, activating it and promoting the transmission of anti-apoptotic signals. AKT1 is often overactivated in lung cancer. The overactivation of AKT1 is closely related to lung cancer cell proliferation, tumor progression, and treatment resistance [17]. JUN is involved in the growth and spread of tumor cells [18]. The activation of the JNK/c-Jun signaling pathway can promote the development and metastasis of lung adenocarcinoma. Moreover, this signaling pathway also interferes with the resistance to radiotherapy in lung cancer [19]. Studies have confirmed that the expression level of HSP90AA1 in tissue samples from lung cancer patients shows an increasing trend, and the related AKT1/ERK signaling pathway also shows enhanced activity. Furthermore, overexpression of HSP90AA1 generally indicates a decrease in survival rate and poor prognosis [20].

Through KEGG pathway enrichment analysis, it was discovered that the "Scutellaria barbata D.Don-Prunella vulgaris L." herb pair may exert anti-lung cancer effects through pathways such as PI3K-Akt and Proteoglycans in cancer, Small cell lung cancer, etc. The PI3K-AKT signaling pathway is a key mechanism in tumor development, and its abnormal activation is a major cause of the formation of various cancers. After PI3K activation, it initiates signal transduction by phosphorylating AKT, promoting cell proliferation, invasion, and metastasis, and enhancing the ability to spread. The PI3K/AKT pathway maintains cell survival by regulating mTOR, FOXO, and GSK3, affecting cell metabolism, growth, and apoptosis. It prevents cell apoptosis by inhibiting pro-apoptotic proteins, aiding cell survival, and tumor cells rely on this mechanism to evade apoptosis and achieve unlimited proliferation [21]. Researching and developing inhibitors targeting this pathway has become a hot topic in the development of anti-cancer drugs. And multiple core active components of the "Scutellaria barbata D.Don-Prunella vulgaris L." herb pair have been mentioned to inhibit this pathway. The results of molecular docking also indicate that AKT1 has strong binding activity with multiple ligands, especially with quercetin and baicalein. HSP90AA1 also interacts with key proteins of the PI3K-Akt pathway, maintaining their structural and functional stability. Its molecular docking results with kaempferol also rank high, and these results all indicate that the main active components can regulate key targets and pathways.

This study employed network pharmacology methods to explore the potential mechanism of action of the "Scutellaria barbata D.Don-Prunella vulgaris L." herb pair in the treatment of lung cancer. The results indicated that the herb pair, through various active components including quercetin, affects key targets such as TP53 and AKT1, thereby regulating signal transduction pathways like PI3K-Akt, and impacting cell proliferation, apoptosis, and invasion / metastasis. The findings suggest that traditional Chinese medicine may enhance treatment efficacy by the synergistic

effects of multiple components targeting multiple targets and pathways. Additionally, the application of molecular docking technology verified the favorable interaction between the core components and their targets, providing valuable reference information for more in-depth drug development in lung cancer treatment.

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