Investigate the Mechanism of Alkaloids of Sophora Flavescens for Co-Treatment of Lung Cancer and Liver Cancer

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Abstract: *Background:* Both lung cancer and liver cancer are malignant tumors worldwide. In clinical practice, co-morbidities of lung cancer and liver cancer are not uncommon. As lung cancer and liver cancer share some common pathways in their pathogenesis, the research on co-treatment drugs for the two cancers is needed to explore potential therapeutic agents. *Case information:* Alkaloids of Sophora flavescens contains active ingredients and exhibits antitumor activities, but its synergistic mechanism has not been elucidated. In the present study, network pharmacology was used as a powerful tool to investigate the potential mechanism of Alkaloids of Sophora flavescens in the co-treatment of lung cancer and liver cancer. *Discussion:* In Alkaloids of Sophora flavescens, a total of thirteen alkaloids could co-treat lung and liver cancers by acting on 223 key targets, in which five core targets were obtained in both lung cancer and liver cancer based on the network pharmacology approach. There were six alkaloids which had a greater effect on the two cancers. The biological processes involved 10 biological processes, 10 cellular components and 10 molecular functions. *Conclusion:* The present study revealed the potential mechanism and provided a systematical theoretical basis for clinical application of Alkaloids of Sophora flavescens in the co-treatment of lung cancer and liver cancer.

Keywords: Alkaloids of Sophora Flavescens; Lung Cancer; Liver Cancer; Core Target

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1 Introduction

Lung cancer and liver cancer are the most common malignant tumors worldwide, posing a serious threat to human health. Lung cancer ranks first among malignant tumors in terms of morbidity and mortality, and its causative factors include smoking, environmental pollution, and genetic susceptibility, etc [1, 2]. Liver cancer, especially primary liver cancer, is in the forefront malignant tumors in terms of morbidity, and the main causative factors include hepatitis B virus infection, cirrhosis, and aflatoxin exposure, etc [3, 4]. Due to its highly invasive nature and the difficulty of early diagnosis, the majority of patients have already progressed to the middle or late stage when diagnosed, and the prognosis is poor [5, 6]. Despite the remarkable progress of targeted therapy and immunotherapy in recent years [7, 8], the clinical efficacy of these two cancers still faces great challenges due to tumor heterogeneity and drug resistance. In clinical practice, co-morbidities of lung cancer and liver cancer are not uncommon, but the strategy for co-treatment of the two diseases is still in the exploratory stage. For patients with

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co-morbidities, a single therapeutic regimen is often difficult to achieve ideal efficacy, and a combination of therapeutic strategies should be adopted according to the situation. It is worth noting that lung cancer and liver cancer share some common pathways in their pathogenesis, such as aberrant activation of signaling pathways such as EGFR, VEGF, and PI3K/AKT, which provides a theoretical basis for exploring the synergistic treatment strategy for multiple cancer types. However, the research on co-treatment drugs for the two cancers is still limited, and there is an urgent need to explore potential therapeutic agents with both multi-target effects and low toxicity.

Alkaloids of Sophora flavescens are an important class of alkaloid mixture extracted from the traditional Chinese medicine Kushen (*Sophora flavescens*), which mainly contains active ingredients such as matrine and oxymatrine [9-11]. In recent years, it has been found that alkaloids from Sophora flavescens exhibit broad activities in the antitumor field, especially in the treatment of liver cancer and lung cancer, and have been used as adjuvant therapy for a variety of cancers. Experimental studies have demonstrated that alkaloids from Sophora flavescens can exert antitumor effects through mechanisms such as inhibiting tumor cell proliferation, inducing apoptosis, blocking the cell cycle and modulating the immune microenvironment [12]. However, the current studies on the antitumor mechanism of alkaloids from Sophora flavescens mostly focus on a single component or a single cancer species. Neither systematic study on Alkaloids of Sophora flavescens nor synergistic mechanism of its multicomponent-multitarget-multipathway has been elucidated yet. As an emerging research method, network pharmacology can analyze the complex relationship between drugs and diseases from the perspective of systematic biology [13-15]. In the present study, we used network pharmacology as a powerful tool to investigate the potential mechanism of Alkaloids of Sophora flavescens in the co-treatment of lung cancer and liver cancer, in order to provide a more in-depth theoretical basis for clinical application.

2 Data Sources and Methods

2.1 Data Sources

Several relevant databases were used in this study. SuPred (https://prediction.charite.de/index.php) was used for collecting information on the target of a compound, SwissTargetPrediction (http://swisstargetprediction.ch/) was used for collecting targets of action for alkaloids, OMIM (https://www.omim.org/) was used for collecting disease targets, GeneCards (http://tcm.cmu.edu.tw/index.php) was used for collecting disease targets, DisGeNET (https://www.disgenet.org/) was used for collecting disease targets, String (https://cn.string-db.org/) was used for collecting protein-protein interaction information, PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used for collecting compound structures and targets of action, Uniprot (https://www.uniprot.org/) was used for collecting target protein sequence information and PDB (https://www.rcsb.org/) was used for collecting protein structures.

2.2 Softwares

The relevant softwares used in this study were listed as follows. AutoDock Vina (https://vina.scripps.edu/) was used for molecular docking, CytoScape 3.8.0 (https://cytoscape.org/) was used for visualization of network pharmacology, and Pymol 2.5.2 (https://pymol.org/2/) was used for visualization of molecular docking results.

2.3 Methods of Analysis

2.3.1 Collection of Alkaloid Action Targets

In our previous studies, a total of 14 alkaloids were identified from Alkaloids of Sophora flavescens, including 5α, 9α-dihydroxymatrine, 12α-hydroxyl oxysophocarpine, 12α-hydroxysophocarpine oxysophoridine, oxysophocarpine, oxymatrine, 9α-hydroxysophocarpine, N-methylcytisine, sophoramine, anagyrine, sophoridine, lupinine, matrine and lehmannine. Alkaloid action targets were collected from SuPred database and SwissTargetPrediction database.

2.3.2 Collection of Disease Targets

Disease-related targets were collected from GeneCards, OMIM and DisGeNET disease databases by using “Lung cancer” and “Liver cancer” as keywords. The results from above three databases were combined into a union dataset.

2.3.3 Screening of Disease Targets

The Venny 2.1.0 online platform was used to obtain

common targets for two diseases, lung cancer and liver cancer.

2.3.4 Construction of Venn Diagrams

Use the Microbiotics online platform (http://www.bioinformatics.com.cn/) to obtain Venn diagram of alkaloid targets and disease-intersecting targets.

2.3.5 Construction of PPI Network and Screening of Core Targets

String database was used to construct the protein protein interaction (PPI) network, and CytoScape 3.8.0 software was used to visualize and analyze the network topology of the PPI network in order to screen the core targets.

2.3.6 Construction of "Component-Target-Disease" Network

CytoScape 3.8.0 software was used to construct the "component-target-disease" network.

2.3.7 Enrichment Analysis of Key Target Genes

GO and KEGG enrichment analysis of key targets was completed by using the DAVID database, and graphs were finished by the use of Microbiotics online platform (http://www.bioinformatics.com.cn/).

2.3.8 Molecular Docking of Alkaloids and Core Targets

Firstly, the sdf file of alkaloids and the pdb format file of core target proteins were obtained from PubChem database and PDB database respectively, and then all-atom docking was carried out after preprocessing using Autodock Vina software. Finally, the docking results were visualized using Pymol software.

3 Results and Discussion

3.1 Sorting of Alkaloid Action Targets

Firstly, PubChem database was used to retrieve the SMILES characters of alkaloids, while ChemDraw software was used to draw the structural formulas in sdf format of 5α, 9α-dihydroxymatrine, 12α-hydroxyl oxysophocarpine and 12α-hydroxysophocarpine. Subsequently, SuPred and SwissTargetPrediction databases were used to predict the action targets of alkaloids, and a total of 314 targets were obtained after de-emphasis.

3.2 Screening of Disease Targets

Firstly, we collected disease-related targets from GeneCards, OMIM and DisGeNET disease databases using "Lung cancer" and "Liver cancer" as the keywords, and used the UniProt KB function to batch convert the gene names into corresponding Uniprot ID numbers after de-duplication. The Uniprot KB function of UniProt database was used to batch convert the gene names into corresponding Uniprot ID numbers, and a total of 13,351 lung cancer-related targets and 8,575 liver cancer-related targets were obtained after de-emphasis. Next, the Venny 2.1.0 online platform was used to obtain the common targets of the two diseases as shown in Figure 1, and a total of 7,493 disease-intersecting targets were obtained.

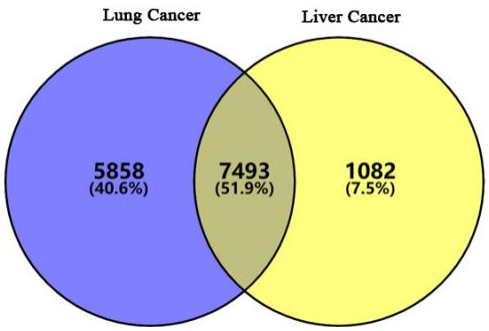

Figure 1 Venn plot of intersecting targets in liver and lung cance

3.3 Construction of PPI Network and Screening of Core Targets

Firstly, the intersecting targets of alkaloid action targets and disease targets (liver & lung cancer) were obtained using the online platform of Microbiotics (http://www.bioinformatics.com.cn/) as shown in Figure 2, in which a total of 223 intersecting target genes were obtained.

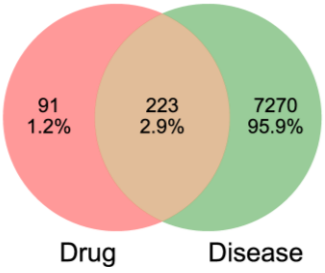

Figure 2 Venn plot of drug-disease intersection targets

Next, 223 key target genes were entered into the String database to construct a protein protein interaction (PPI) network, and the species was selected as "Home sapiens", and the threshold was set to "highest confidence". Then, the result file in CSV format was downloaded and CytoSape 3.8.0 software was used to visualize the PPI network as shown in Figure 3, which has 219 nodes and 2019 edges. Nodes represent different gene proteins and edges represent proteins whose connected proteins have interaction relationships. The size and color of the nodes reflect the size of the degree value. The top 5 targets are STAT3, HSP90AA1, HIF1A, HSP90AB1 and NFKB1, with degree values of 93, 83, 73, 72 and 71, respectively.

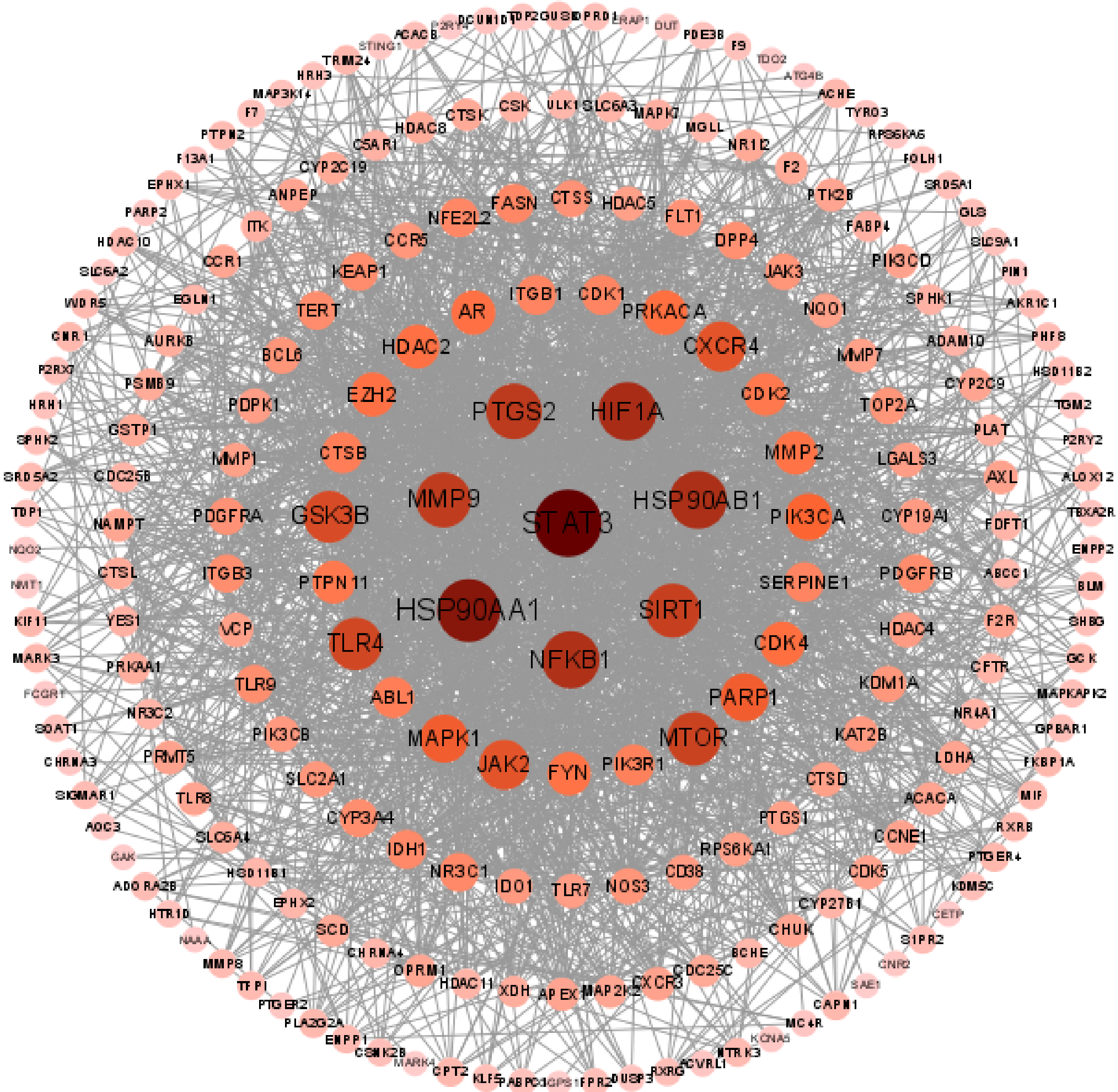

Figure 3 PPI networks for drug-disease intersection targets

3.4 Construction of "Component-Target-Disease" Network

CytoScape 3.8.0 software was used to construct the "component-target-disease" regulatory network as shown in Figure 4, in which green nodes represent key targets, purple nodes represent alkaloids and red nodes represent 2 diseases. The constructed network has 238 nodes and 1821 edges while no data available for 12α-hydroxyl oxysophocarpine. In addition, it can be seen that 13 alkaloids act on 223 key targets for the treatment of two diseases, liver cancer and lung cancer.

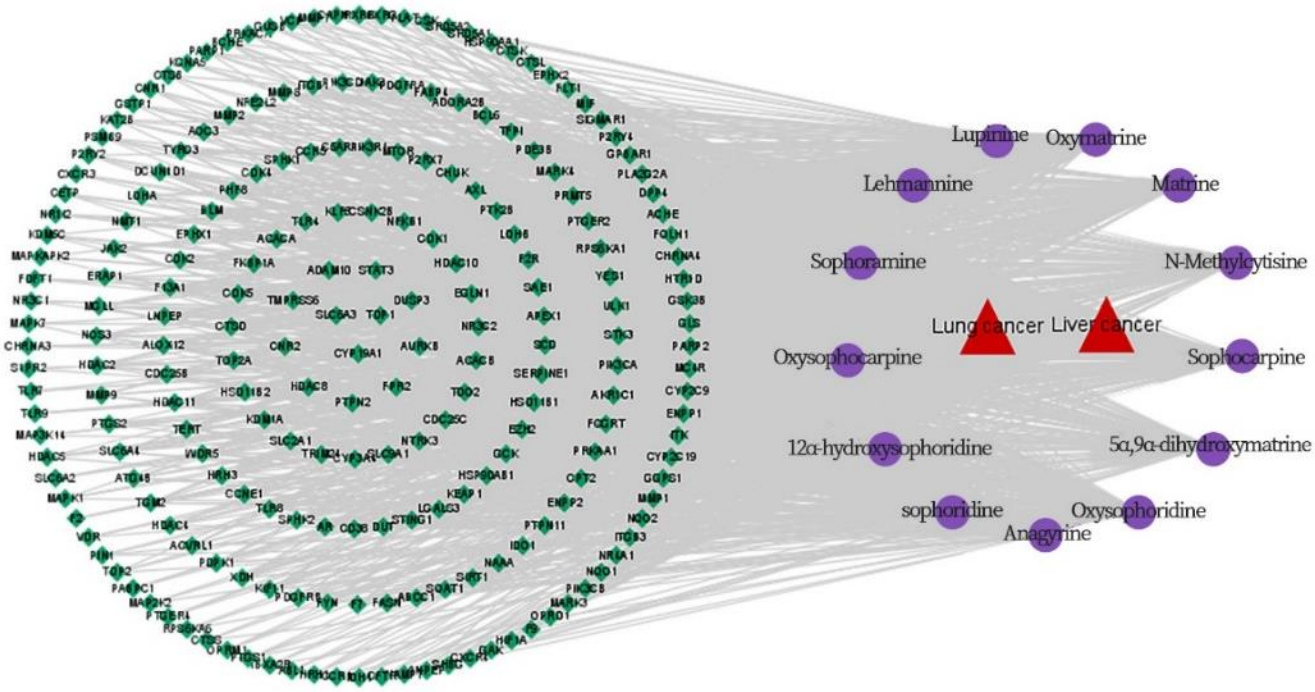

Figure 4 "Component-target-disease" regulatory network

3.5 Enrichment Analysis of Key Target Genes

In order to study the specific roles of key target proteins of alkaloids in biological processes, 223 key target genes were enriched using the DAVID database (https://david-d.ncifcrf.gov/). Firstly, the gene information of "Homo sapiens" was selected, and the entries of Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) were chosen. The entries related to biological process (BP), cellular component (CC) and molecular function (MF) were selected, and "P<0.05" was used as the filtering condition. Then, the entries with the top 10 enrichment scores for each type were selected and analyzed by using the Microbiotics online platform to make graphs as shown in Figure 5. The results showed that Alkaloids of Sophora flavescens treat liver and lung cancers by acting on key targets such as TDP1, CYP19A1, TRIM24, KDM1A, HSD11B2, CTSD, CDK5, and FKBP1A. The biological processes involved 10 biological processes (BP), 10 cellular components (CC) and 10 molecular functions (MF). The 10 biological processes (BP) include inflammatory response, response to lipopolysaccharide, response to lipopolysaccharide, protein phosphorylation, positive regulation of cytosolic calcium ion concentration and so on. The 10 cellular components (CC) are macromolecular complex, extracellular exosome, cytoplasm and so on. The 10 molecular functions (MF) include kinase activity, protein binding, protein tyrosine kinase activity and so on.

In order to further investigate the mechanism of alkaloid-regulated signaling pathways, we used the DAVID database to analyze the KEGG enrichment of 223 key targets. The gene information of "Homo sapiens" was selected, and the entries with the top 30 enrichment scores were chosen and analyzed by using the Microbiotics online platform. As shown in Figure 6, the main pathways of alkaloids in the treatment of lung and liver cancers are PI3K-AKT signaling

pathway, HIF-1 signaling pathway, pathways in cancer, insulin resistance, apoptosis and so on. In addition, it revealed that the pathway in cancer was the pathway with the most targets which contained 44 key targets.

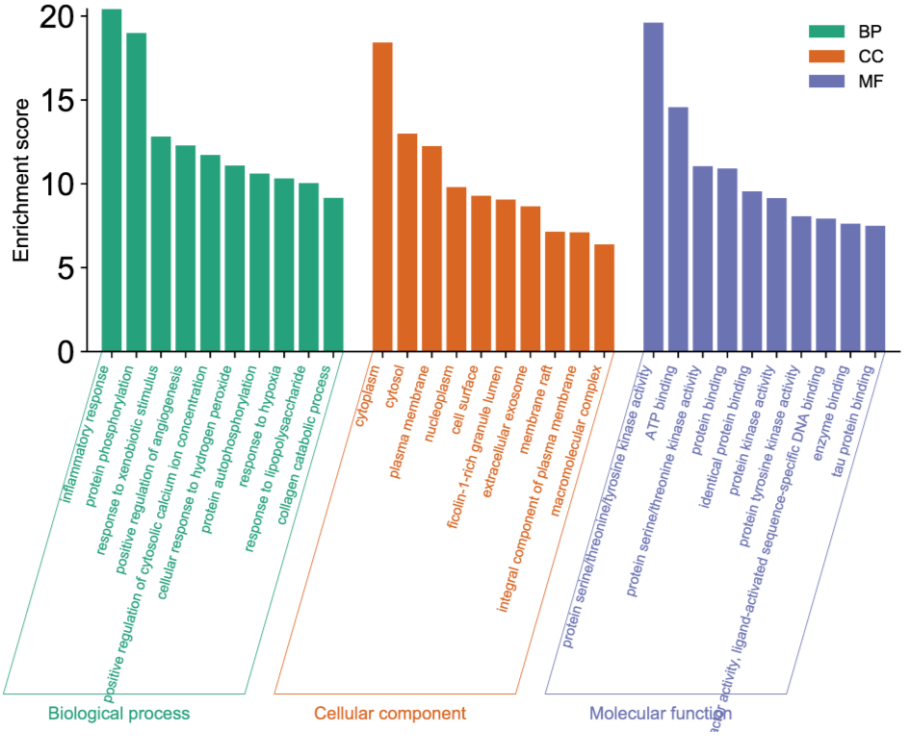

Figure 5 GO enrichment analysis of key targets

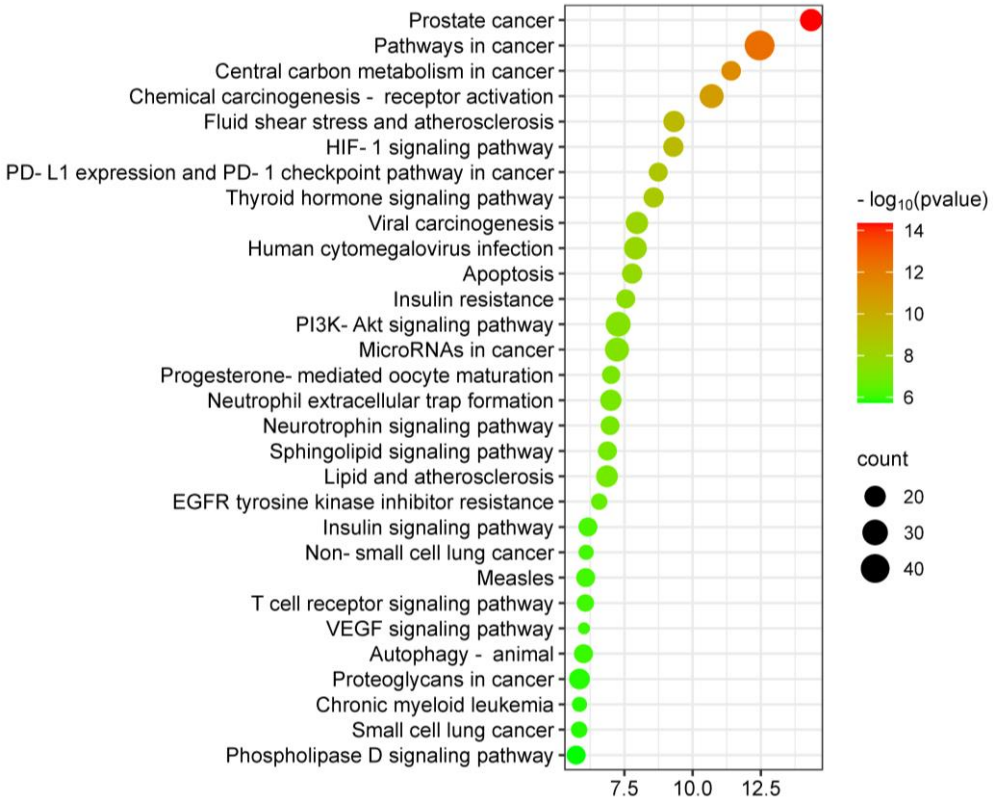

Figure 6 KEGG pathway enrichment analysis of key targets

3.6 Molecular Docking of Core Targets

Molecular docking model was constructed to examine the interaction of 13 alkaloids with five core target proteins. The first step was compound structure preprocessing. Sdf files of alkaloids were obtained from PubChem database/ChemDraw software, and energy minimization, hydrogenation and charge correction were performed on the compounds using Autodock Vina software. The second step was protein structure preprocessing. The pdb format file of the core target protein was downloaded from the PDB database (htp: //www.rcsb.org/pdb), and the corresponding PDB IDs of the target proteins are shown in Table 1. Then Autodock Vina software was used to perform preprocessing of the protein structure, including water molecules in the crystalline structure of proteins, protein inhibitors and salt ions required for crystallization, repairing missing hydrogen atoms on protein residues, and conformational optimization of the protein. The third step was molecular docking. Autodock Vina software was used to carry out molecular docking, with the compound as the ligand and the protein as the receptor. All-atom docking was chosen as the docking method, and the top 10 conformations in the docking score were output.

Table 1 PDB ID of the core target protein

No.	core target protein	PDB ID
1	STAT3	6NJS
2	HSP90AA1	3O0I
3	HIF1A	1H2K
4	HSP90AB1	1UYM
5	NFKB1	2DBF

The presence of high expression and activity of STAT3 is usually associated with poor clinical prognosis, and is regarded as one of the important factors in tumor diagnosis and treatment. HSP90AA1 and HSP90AB1 are two isoforms of heat shock protein HSP90, which is a biomarker of cellular stress response and an important intracellular chaperone protein. Hypoxia-inducible factor-1 is an oxygen-dependent transcription factor. It could mediate the expression of hypoxia-associated genes to facilitate cell proliferation and angiogenesis. Nuclear factor-κB is a key player in innate immunity, inflammation, cell survival, proliferation as well as apoptosis. The predictions in the present study were consistent with previous literature.

The binding between alkaloids and core targets was comprehensively evaluated based on the docking score S and the number of intermolecular interaction bonds. The docking score S (unit: -kcal/mol) represents the binding ability between compounds and proteins. The smaller S is, the more stable the compounds are bound to proteins. The more interactions such as the formation of hydrogen bonds between molecules indicate the stronger binding of the compound to the protein. As shown in Table 2, the binding free energy of matrine to the HIF1A protein is -5.27 kcal/mol, oxysophocarpine to the HSP90AA1 protein was -5.78 kcal/mol, oxymatrine to the HIF1A protein was -5.11 kcal/mol, oxymatrine to HSP90AA11 is -5.76 kcal/mol, 12α-hydroxyl oxysophocarpine to HIF1A was -5.36 kcal/mol, sophoridine to HIF1A is -5.39 kcal/mol, 5α, 9α-dihydroxymatrine to STAT3 is -5.55 kcal/mol and oxymatrine to STAT3 is -5.41 kcal/mol.

Table 2 PDB ID of the core target protein

Compound	Core targets	Docking score (s)
oxymatrine	HSP90AA11	-5.76 kcal/mol
12α-hydroxyl oxysophocarpine	HIF1A	-5.36 kcal/mol
sophoridine	HIF1A	-5.39 kcal/mol
5α, 9α-dihydroxymatrine	STAT3	-5.55 kcal/mol
oxymatrine	STAT3	-5.41 kcal/mol
matrine	H1F1A	-5.27 kcal/mol
oxysophocarpine	HSP90AA11	-5.78 kcal/mol
oxymatrine	H1F1A	-5.11 kcal/mol

4. Conclusion

Five core targets including STAT3, HSP90AA1, HIF1A, HSP90AB1 and NFKB1 were obtained in both lung cancer and liver cancer based on the network pharmacology approach. In Alkaloids of Sophora flavescens, 13 alkaloids could co-treat lung and liver cancers by acting on 223 key targets and 6 alkaloids such as matrine, oxysophocarpine, oxymatrine, sophoridine, 5α, 9α-dihydroxymatrine and 12α-hydroxysophocarpine had a greater effect on the two cancers.

Competing Interest

The authors declare no competing interests.

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