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Mechanisms of Action of the Herba Salviae Chinensis-Fructus Akebiae Pair in the Treatment of Non-small Cell Lung Cancer based on a Network Pharmacology Study

Guomin Dong[1], Zhiyun Zhang[1], Qian Ge[3], Pengyu Han[1], Jian Jiang[1], Suoni Li[2], Jiequn Ma[2], Jie Bai[2], Yafen Qi[1], Zheng Zhao[1,2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
[2]Shaanxi Provincial Cancer Hospital, Xi'an 710000, Shaanxi, China
[3]Xi'an Medical University, Xi'an 710021, Shaanxi, China
Correspondence Author

Abstract: ***Objective**: To study the active ingredients and mechanism of action of "Herba Salviae Chinensis-Fructus Akebiae" drug pair in the treatment of non-small cell lung cancer (NSCLC) by using network pharmacology to provide theoretical basis for clinical application. **Methods**: The TCMSP (Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform) was used to obtain the active ingredients and targets of "Herba Salviae Chinensis-Fructus Akebiae", and the results were analyzed by the Genecard (https://genecard, org/) database, the Online Mendelian Inheritance Network (OMI), the Genecard database, the Genecard database, and the Online Mendelian Inheritance Network. We searched the Genecard (https://genecard, org/) database and the Online Mendelian Inheritance in Man (OMIM) database (http://omim. org/) for non-small cell lung cancer (NSCLC) disease targets by selecting "NSCLC" as a keyword, and then obtained the results of the "Herba Salviae Chinensis-Fructus Akebiae" drug pair by using venn. We used venn to obtain the intersecting targets of the drug pair "Herba Salviae Chinensis-Fructus Akebiae" and NSCLC, and then used the STRING database and Cytoscape to construct a common target visualization network diagram. The gene (GO) enrichment analysis and KEGG enrichment analysis were realized with the help of metascape database and Microbiology platform. **Results**: Five active ingredients of Ishiminokan-Precipitant were obtained, and 3695 non-small cell lung cancer targets and 123 common targets were obtained. GO analysis showed that the process of GO enrichment was mainly related to the regulation of responses to inorganic substances, transcriptional regulatory complexes, cytokine receptor binding, etc. KEGG enrichment showed that the process of KEGG enrichment was mainly through Pathways in Cancer, ILRT, and cytokine receptor binding. KEGG enrichment showed that KEGG mainly exerted anti-cancer effects through pathways in cancer and IL-17 signaling pathway. **Conclusion**: Using the method of network pharmacology, it is proved that the drug pair "Herba Salviae Chinensis-Fructus Akebiae" can exert therapeutic effects on NSCLC through multi-components, multi-targets, and multi-pathways, and provides the theoretical basis for the clinical application.*

Keywords: Herba Salviae Chinensis, Fructus Akebiae, Non-small cell lung cancer, Network pharmacology.

1. Introduction

Lung cancer is the most common malignant tumor with the highest morbidity and mortality rates [1]. According to the histology of cancer cells, lung cancer can be divided into two types: small cell carcinoma (SCLC) and non-small cell carcinoma (NSCLC), which is the most common subtype and accounts for 85-90% of all lung cancers, and consists of various histologic subtypes, including lung adenocarcinoma, squamous carcinoma, and large-cell lung carcinoma [2]. In addition to surgery, chemotherapy, radiotherapy, targeted therapy and immunotherapy, traditional Chinese medicine (TCM) has unique advantages in preventing tumor development, reducing toxicity and enhancing efficacy, and reducing tumor recurrence and metastasis [3]. Currently, the mainstream view is that blood stasis is common in tumor patients, and the method of activating blood circulation and removing blood stasis can inhibit tumor growth, improve the hypercoagulability of blood, inhibit invasion and metastasis, prolong the survival time of the patients, and improve the quality of life [4]. Since NSCLC involves many mutated genes, it cannot be effective by intervening a single target. Therefore, the mode of thinking in scientific research has changed from a single causal relationship to the interaction between multiple factors and the network formed by such interaction, especially in the case of multi-component, multi-target and multi-pathway traditional Chinese medicines. Herba Salviae Chinensis(SCH), also known as purple ginseng and small red ginseng, is cool in nature, bitter, pungent, and flat in taste, and has the efficacy of activating blood circulation and removing blood stasis, clearing heat and removing toxins, and promoting the circulation of qi to dissipate knots, which is documented in Compendium of Materia Medica, and **Suzhou Herbs** and **Medicinal Materials of Local Origin** [5]. Researchers have extracted a variety of active ingredients from SCH, such as polysaccharides, triterpenoids, polyphenols, etc. Experiments have proved that Fructus Akebiae (AF) extracts can act on a variety of cancer cell lines, inhibit the proliferation of cancer cells and induce apoptosis, inhibit tumor angiogenesis, and regulate the immune function. It is cold in nature, bitter in taste, and has the effects of dispersing the liver, regulating qi, activating blood circulation, relieving pain and dispersing knots. As a traditional Chinese medicine in China, it has been recorded in **Rihuazi Materia Medica** and **Kaibao Materia Medica**. The compounds isolated from the herb are mainly triterpenes, their saponins and amino acids, which have a wide range of biological activities, with significant antibacterial, antitumor and hepatoprotective effects, and are commonly used in traditional Chinese medicine for preventing

hepatocellular carcinoma, resolving blood stasis and promoting the flow of qi, as well as having certain clinical efficacy in gastric cancer, hepatocellular carcinoma, lung cancer, and other cancerous diseases [6]. The combination of the two herbs can enhance the efficacy of AF in removing blood stasis, so as to make the power of removing blood stasis and dispersing knots more powerful, while AF can strengthen the power of SCH in activating blood circulation and promoting qi circulation, so as to make the qi and blood operate as usual. When used together, they can activate Blood, move Qi, resolve stasis and disperse knots. Prof. Qi Yuanfu often used this medicine in the treatment of lung cancer patients with stagnant qi and uncomfortable emotions [7]. Since the treatment of diseases by TCM is characterized by multiple components, multiple targets, and multiple pathways, the study of the biological mechanism of TCM in treating diseases needs to be holistic and systematic. Network pharmacology is a new approach to study the interactions among active compounds, targets and diseases of TCM, which is essentially a systems biology based on the mining of big data. This method is scientific and visualized for the evaluation of drug efficacy and the study of the mechanism of action. In this study, we investigated the potential mechanism of NSCLC treatment by analyzing the targets and signaling pathways of the active compounds of the "SCH-AF" drug pair.

2. Data and Methods

2.1 The keywords "SCH-AF" were entered into TCMSP (TCMSP, http:// tcmspw.com/tcmsp.php), and the compounds corresponding to the constituents were found.

2.2 Screening of active ingredients and target proteins Oral bioavailability (OB) is one of the most important pharmacokinetic ADME parameters, which refers to the speed and degree of absorption of the active ingredients into the human blood circulation, and the lower the OB value, the worse the therapeutic effect of the drug may be. The lower the OB value, the worse the therapeutic effect of the drug may be. Drug-like properties (DL) refers to the degree of similarity between a compound and a known drug, and usually the lower the DL value, the lower the possibility that the compound can be used as the active ingredient of the drug. Therefore, OB ≥ 30% and DL ≥ 0.18 were used as criteria for further screening of ingredients with high biological activity. The ingredients were matched to drug targets in TCMSP, and the obtained targets were corrected to standard gene names by Uniprot database (https:// www.uniprot.org/).

2.3 Disease targets were searched and screened by Genecard (https://genecard, org/) and Online Mendelian Inheritance in Man (OMIM) databases (http://omim.org/) using the keyword "NSCLC". Disease targets were searched and screened. Duplicate targets were removed to obtain known targets. The R language was used to match and map the targets related to the active ingredients of the drugs with the disease targets, and a Venn diagram was plotted to obtain the potential anti-NSCLC targets of the active ingredients of "SCH-AF".

2.4 The STRING database platform (https://stnng-db. org/Version 10.5) was used to predict protein interactions. The intersecting targets of the anti-NSCLC effects of "SCH-AF" were imported into the STRING database, and human (Homo sapiens) was selected as the study species to obtain the protein interactions, and the results were exported in TSV format. The light blue line represents the protein interactions from the database, and the purple line represents the experimentally verified protein interactions.

2.5 Constructing the "drug-component-target-disease" network Cytoscape (Version3.8.0) is used to construct the "drug-component-target-disease" network diagram. The core targets of "SCH-AF" against NSCLC were obtained by using the values of Betweenness, Closeness and Degree to sort and remove the free targets. The core target STRING bubble diagrams were then imported into Cytoscape (Version3.8.0) in TSV format, in which the nodes represent the active ingredients of "SCH-AF" against NSCLC, key target genes and NSCLC, and edges represent the active ingredients of Ishizumi Prednisolone against NSCLC. "Edge represents the interaction relationship between "SCH-AF" drug pair and corresponding active ingredients, active ingredients and target genes, and disease and target genes.

2.6 Constructing protein-protein interaction (PPI) networks Using the STRING platform (https://string-db.org/), we constructed intersecting target PPI networks, and ranked them by Degree value in order to identify the effects of the main active ingredients in the Ishimitsu-Prezumi pairs on NPD. The PPIs were ranked by Degree value to identify the key targets of the main active ingredients in the "SCH-AF" drug pair on NSCLC.

2.7 Pathway enrichment analysis The acquired core genes were imported into the Metascape database (https:// metascape.org/) for gene ontology (GO) biological function enrichment analysis and Kyoto Encyclopedia of Genes and Ge nomes (KEGG) signaling pathway enrichment analysis. Signaling pathway enrichment analysis. The results were plotted as bubble charts and bar charts with the help of the microinformatics platform (bioinformatics.com.cn).

3. Results

3.1 The TCMSP database was used to search for the active ingredients of SCH and AF, and a total of 29 chemical constituents were obtained, of which 17 were SCH and 12 were AF. 2 active ingredients of SCH and 3 active ingredients of AF were finally screened out with the screening conditions of OB ≥30% and DL≥0.18. See Table 1.

Table 1: Values of active ingredients, oral bioavailability and drug-like properties of the drug "SCH-AF".

herbs	ingredient name	MOL ID	OB(%)	DL	herbs	ingredient name	MOL ID	OB(%)	DL
SCH	beta-sitosterol	MOL000358	36.91	0.75	AF	glyceryl linolenate	MOL010929	38.14	0.31
	quercetin	MOL000098	46.43	0.27		sitosterol	MOL000359	36.91	0.75
						2-Monoolein	MOL008121	34.23	0.29

3.2 Acquisition of drug targets A total of 162 targets were obtained from the target screening of five active compounds by TCMSP.

3.3 Acquisition of NSCLC disease targets The Genecard (https://genecard,org/) database and Online Mendelian Inheritance in Man (OMIM) database (http://omim.org/) were used to search and screen the disease targets with the keyword "NSCLC". The keyword "NSCLC" was used as the keyword to search and screen the disease targets. 3320 targets obtained from Genecard database were retained after taking the median twice and added with 469 targets obtained from OMIM database, and 3695 disease targets were obtained after deleting the duplicates. The R language was used to match and map the targets related to the active ingredients of the drugs with the disease targets, and a Venn diagram was plotted as shown in Figure 1, and the potential anti-NSCLC targets of the active ingredients of SCH and AF were obtained.

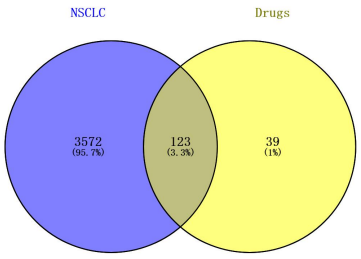

Figure 1: Venn diagram

3.4 Acquisition of common targets and construction of PPI network In order to further clarify the common targets of the "SCH-AF" drug pair and NSCLC, the intersection part of the venny diagram was acquired, and the PPI network was constructed by inputting 123 intersecting target proteins into the String platform, and the confidence level was set as Highest≥0.4. Highest≥0.4 to construct the PPI network. See Figure 2.

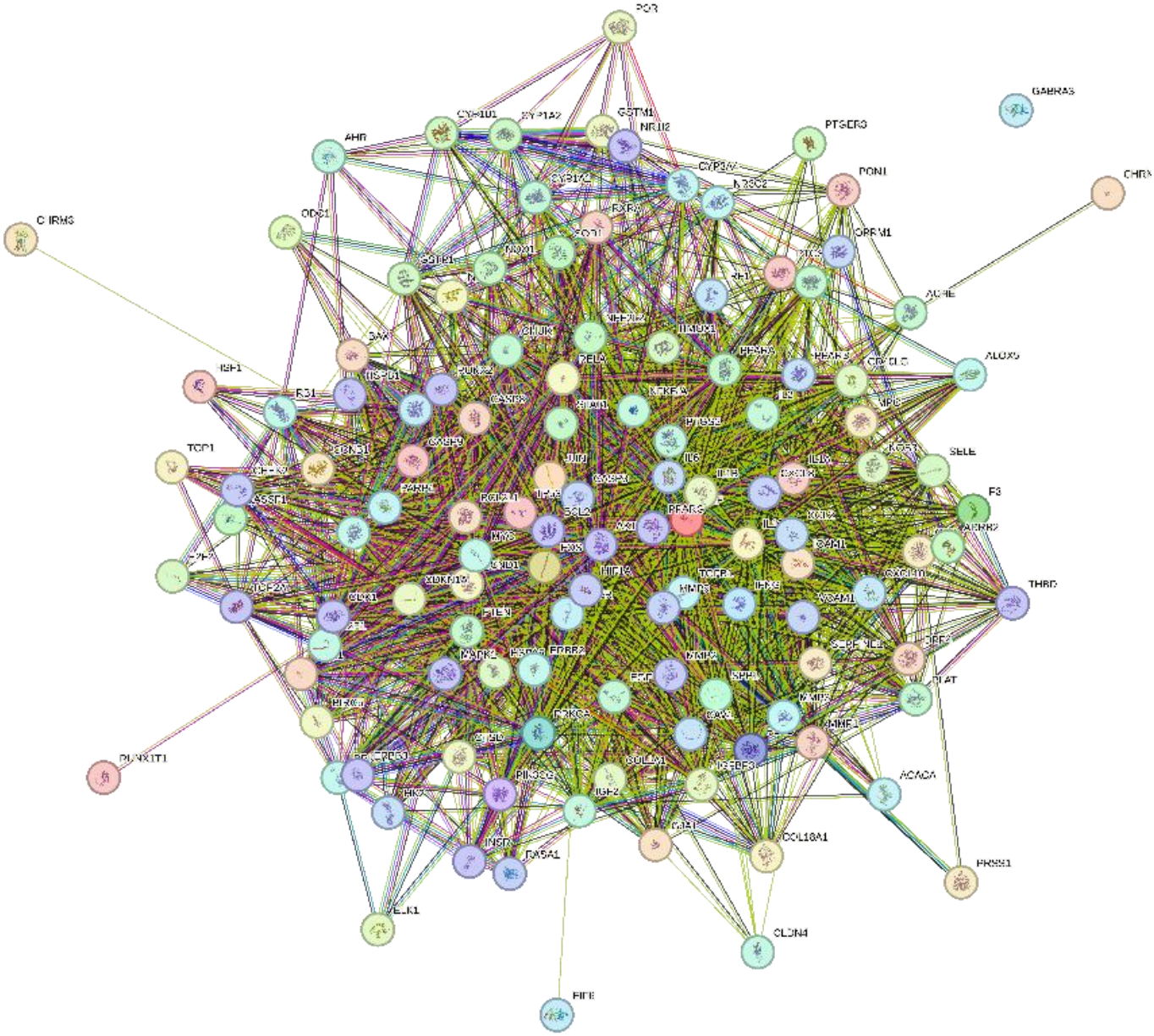

Figure 2: PPI protein interaction network diagram

The PPI network was imported into Cytoscape software and sorted by Degree value to obtain 54 core proteins, the specific gene names are shown in Table 2.

Table 2: Core protein gene names

Gene name	Gene name	Gene name	Gene name	Gene name	Gene name
AKT1	PTGS2	ERBB2	ICAM1	CASP9	AR
TP53	BCL2	CCL2	NFKBIA	MAPK1	HSPA5
TNF	MYC	EGF	BCL2L1	NFE2L2	MMP3
IL6	MMP9	IFNG	CASP8	PARP1	NOS3
CASP3	TGFB1	IL10	RELA	PPARA	MPO
EGFR	PPARG	CXCL8	CDKN1A	SPP1	PGR
IL1B	CCND1	HMOX1	STAT1	CAV1	MMP1
JUN	FOS	IL1A	VCAM1	CXCL10	PRKCA
HIF1A	PTEN	MMP2	SERPINE1	CRP	IGFBP3

A larger value of Degree indicates that the corresponding target is more important in the network and its graph is larger, see Figure 3.

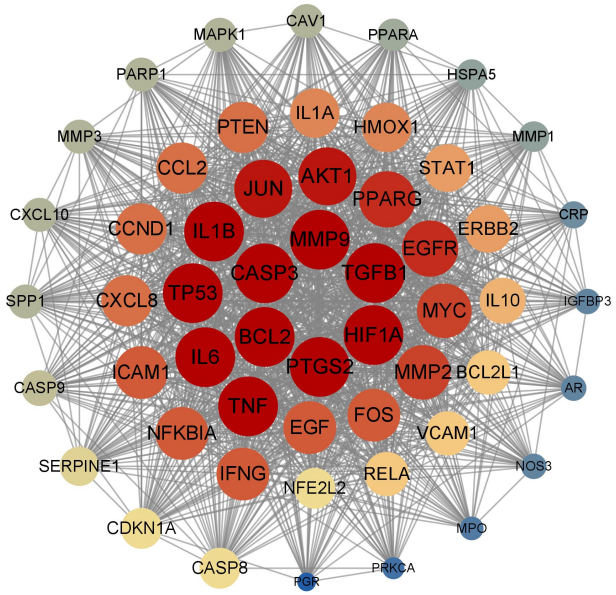

Figure 3: PPI network diagram

3.5 Drug-ingredient-target-disease co-expression network construction

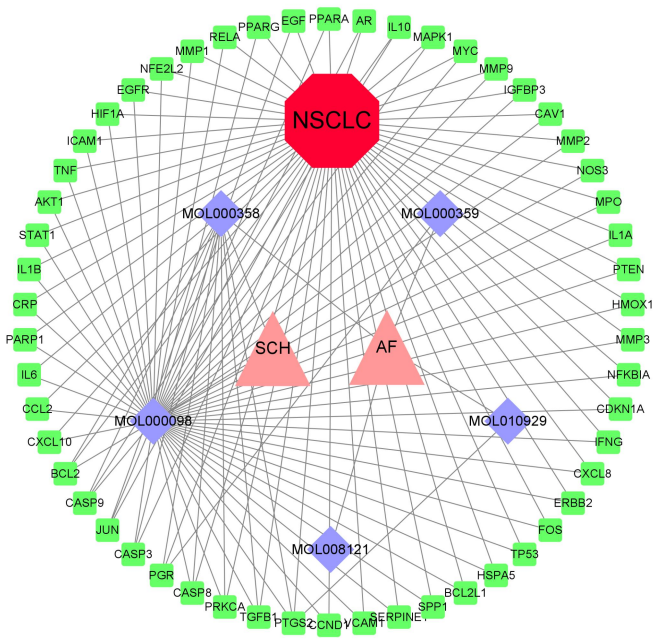

Figure 4: drug-component-target-disease network diagram

The "SCH-AF" drug pairs and their active ingredients, active ingredients and key target genes, key target gene-lung cancer interactions and node attributes were created and imported into Cytoscape to construct the "drug- ingredient- target-disease" network, see Figure 4. The drug- component- target-disease network was imported into Cytoscape to construct the "drug-component-target-disease" network, see Figure 4, in which the top 5 drug components were ranked according to the value of the relationship with NSCLC from high to low: quercetin was 54, beta-sitosterol was 11, sitosterol was 2, glyceryl linolenate was 2, and 2-monoolein was 1. Monoolein was 1. See Table 3.

Table 3: Top 5 active ingredients of drugs with Degree value

active ingredient	Degree value	Source Chinese Medicine
quercetin	54	SCH
beta-sitosterol	11	SCH
sitosterol	2	AF
glyceryl linolenate	2	AF
2-Monoolein	1	AF

3.6 GO enrichment analysis Using metascape to analyze the GO of the relevant target proteins in three aspects: molecular function, cellular components and biological process, integrating the corrected logP value and enrichment in the function of the gene, respectively, select the top-ranked genes with the help of the microbiology platform for the presentation of the histogram, see Figure 5. The analysis results show that the above proteins are mainly involved in the response to inorganic substances, lipopolysaccharide, and bacterial molecules in biological processes; in cellular components, they are mainly involved in the transcriptional regulatory complex, RNA polymerase II transcriptional regulatory complex, etc.; and in molecular functions, they are mainly involved in the binding of cytokine receptor, the binding of transcriptional co-regulatory factors, and the binding of ubiquitin-like protein ligase. It is hypothesized that "SCH-AF" pair may exert its anti-tumor effect in the treatment of NSCLC through the above functions.

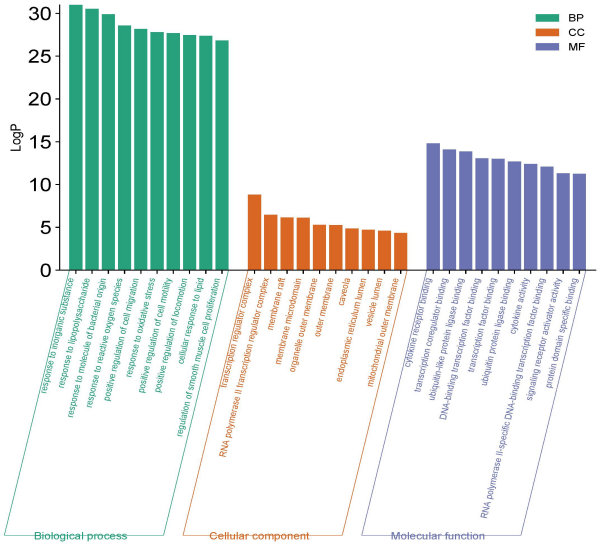

Figure 5: GO enrichment analysis diagram

3.7 KEGG pathway enrichment analysis KEGG analysis was performed on 30 anticancer key genes, see Figure 6. Sorting according to LogP value, the top 20 pathways were selected to be displayed in a bubble diagram, which mainly involved Pathways in cancer,

IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway and other signaling pathways.

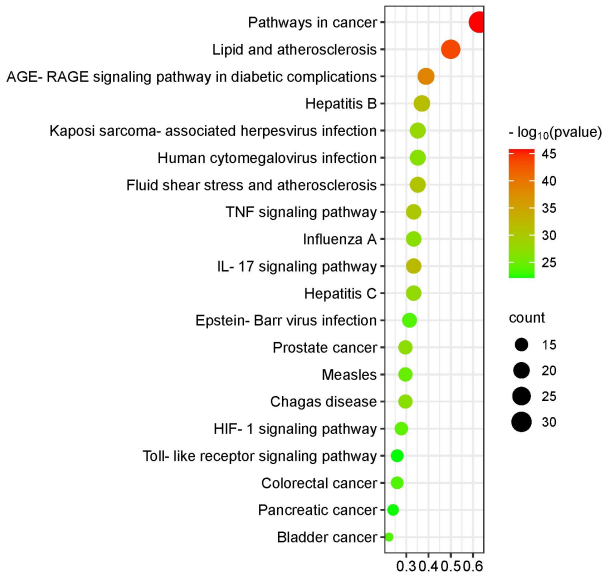

Figure 6: KEGG pathway enrichment analysis diagram

4. Discussion

Lung cancer is one of the most common cancers in the world, with high morbidity and mortality rates [1]. NSCLC is the most common subtype of lung cancer, accounting for 85-90% of all lung cancer types [2].Stage I or II NSCLC is treated with surgical resection of the tumor, supplemented by adjuvant therapy. In contrast, when the disease progresses to stage III or IV, treatment shifts to chemotherapy or radiotherapy [8],[9]. However, almost all conventional chemotherapeutic agents share the same limitations, including nonspecific targeting, low bioavailability, and development of resistance, which limits their efficacy in cancer treatment [10]. So despite all these options, the prognosis remains very poor with a low 5-year survival rate. Therefore, there is an urgent need to seek a paradigm shift in therapeutic approaches [11]. Traditional Chinese medicine (TCM) has unique advantages in preventing tumorigenesis, reducing toxicity and increasing efficacy, and reducing tumor recurrence and metastasis. There is no record of "lung cancer" in ancient literature, but Chinese medicine attributes lung cancer to the category of "lung accumulation" and "resting cardia" through its clinical symptoms, which mainly include coughing and hemoptysis, chest tightness, shortness of breath, fever, weight loss, and so on. The main manifestations are coughing and hemoptysis, chest tightness, shortness of breath, fever, weight loss, with or without compression of neighboring tissues and metastasis of distant organs. According to Chinese medicine, lung cancer is mainly caused by deficiency of positive qi and a variety of pathogenic factors, such as "deficiency", "phlegm", "stasis" and "toxicity". Toxicity". Although the proportion of each pathogenic factor is different in different stages of tumor, the general treatment is to support the positive and replenish the deficiency, dissolve phlegm and promote dampness, activate blood circulation and remove blood stasis, and clear away heat and remove toxins [12]. SCH has the efficacy of removing blood stasis and activating blood circulation, clearing heat and removing toxins, and promoting the circulation of qi and dispersing knots, etc. Researchers have extracted a variety of active ingredients from SCH, such as polysaccharides, triterpenoids, polyphenols, etc. Experiments have proved that SCH extract can act on a variety of cancer cell lines, with the function of inhibiting the proliferation and inducing apoptosis of cancer cells, inhibiting the generation of tumor angiogenesis, and regulating the immune system. AF has the functions of dispersing the liver, regulating qi, activating blood circulation, relieving pain and dispersing knots. The compounds isolated from prehensile in modern research are mainly triterpenoids, their saponins and amino acids, which have a wide range of biological activities, possessing significant antibacterial, antitumor and hepatoprotective effects, and are commonly used in traditional Chinese medicine for preventing hepatocellular carcinoma, resolving blood stasis and promoting circulation of qi, and also have certain clinical efficacy in gastric, hepatic, and lung cancers and other cancerous diseases [6]. In addition, it also has certain clinical efficacy in gastric cancer, liver cancer, lung cancer and other cancers [6]. The chemical isomers of the pair "SCH-AF" also have good inhibitory effects on the growth of various cancer cells, which suggests that the pair "SCH-AF" has a great potential for development as an anticancer drug candidate.

4.1 Core active ingredients of the drug The active ingredients of the "SCH-AF" drug pair were obtained and according to the "drug-component-target-disease" network diagram, the main active ingredients of the "SCH-AF" drug pair, quercetin and beta-sitosterol, were the most highly associated with the anti-NSCLC target in the topological network. According to the "drug-component-target-disease" network diagram, quercetin and beta-sitostero are the compounds with the highest degree of association with the anti-NSCLC target, i.e., the most central nodes, suggesting that these two active ingredients play important roles in the treatment of NSCLC.1) A number of in vitro studies have found that quercetin can improve and enhance the quality of NSCLC treatment. quercetin can improve and regulate many signaling pathways during cancer development. In addition, it plays a key role in apoptosis, survival, angiogenesis, inflammation, and cell cycle [13]. quercetin can inhibit or down-regulate molecules in a variety of signaling pathways through its effects on apoptosis [14]. It was found that treatment of human A-549 cell line with different concentrations of quercetin at 10, 30 and 60 mM affected microfilaments, microtubules and wave protein filaments, as well as inhibited the expression of wave proteins and N-calmodulin (cytoskeletal proteins), which play a role in migration and induced a significant increase in BCL2/BAX-mediated apoptosis [15]. A previous study reported that quercetin could down-regulate IL-6, STAT-3, Bcl2 activity, NF-kB expression, and up-regulate membrane-bound proteins and PI cell populations by inducing mitochondria-mediated apoptosis in A549 cells in NSCLC [16]. Yonghong Wang et al. reported that for the treatment of NSCLC, the combination of quercetin and Yonghong Wang et al. reported that for the treatment of NSCLC, the combination of quercetin and paclitaxel could promote apoptosis. In addition, paclitaxel was not effective in treating Akt and ERK phosphorylation, which was significantly inhibited by the combination of quercetin and paclitaxel [17]. 2) beta-sitosterol is a natural compound with anticancer properties against various cancers. beta-sitosterol

down-regulates the expression of FOXM1 both in vitro and in vivo. overexpression of FOXM1 attenuated the inhibitory effect of beta-sitosterol on HepG2 cells. HepG2 cells. In addition, β-sitosterol inhibited epithelial-mesenchymal transition (EMT) in HepG2 cells, whereas FOXM1 overexpression promoted EMT. mechanistically, β-sitosterol regulated the transcription of target genes associated with proliferation and metastasis of HepG2 cells through downregulation of FOXM1 inhibition of Wnt/β-catenin signaling. β-sitosterol inhibited EMT in HepG2 cells through downregulation of FOXM1 and inhibition of Wnt /β-catenin signaling inhibition through FOXM1 down-regulation and Wnt/β-catenin signaling shows promising potential as a therapeutic candidate to inhibit HCC growth and metastasis [18].

4.2 Key target genes Analysis of the PPI network revealed that MMP9, CASP3, BCL2, PTGS2, HIF1A, and TGFB1 genes have the closest interactions and are at the core of the network, and can be considered as key targets for therapy. 1) MMP-9 As a metalloproteinase, it plays an important role in ECM remodeling, angiogenesis, metastasis, and cancer progression. In lung cancer, vascular endothelial growth factor (VEGF) induces MMP-9 expression and subsequently increases the likelihood of metastasis. Therefore, MMP-9 inhibitors have emerged as potential targets for anticancer drug development [19]. 2) Caspase-3 initiates the division of GSDME in response to various cellular stresses, resulting in the formation of a fragment capable of creating pores in the cell membrane. These openings allow water and ions to enter the cell, leading to cell enlargement and eventual rupture. This form of cell death is known as cellular pyroptosis. Caspase-3 is a key protein in cellular pyroptosis and apoptosis and controls tumor cytotoxicity when activated. Gasdermin proteins activate caspase-3 in order to induce cellular pyroptosis, which has been implicated in tumorigenesis, progression, and therapeutic response.Caspase-3 is a key protein in cellular pyroptosis and apoptosis and controls tumor cytotoxicity when activated. These proteins can be used as therapeutic biomarkers for cancer detection, and their antagonists may be a novel target [20]. 3) Members of the BCL-2 family of proteins constitute key regulators of apoptosis. Abnormal expression of either pro-survival or pro-apoptotic members of the BCL-2 family of proteins promotes tumor progression and renders malignant tumor cells resistant to anticancer therapy. Current studies have provided a detailed understanding of the control of apoptosis and how different subgroups of BCL-2 family proteins interact with each other. Enabling the development of novel anticancer drugs, such as BH3 mimetics, which can directly activate the apoptotic mechanism by inhibiting pro-survival BCL-2 proteins. These compounds have shown some efficacy in preclinical studies and some of them have entered clinical trials for cancer treatment, among which the BCL-2 specific inhibitor vinatocet FDA has been approved for clinical use [21]. 4) Prostaglandin-endoperoxide synthase 2 (PTGS2), a rate-limiting epoxide synthase that can be induced by inflammatory stimuli, is essential for the production of inflammatory prostaglandins, and has fundamental activities ranging from normal development to human disease Studies have shown that PTGS2 is involved in cancer development, progression, and metastasis Elevated PTGS2 is associated with enhanced angiogenesis, increased tumor invasion, and decreased apoptosis, and thus PTGS2 inhibitors have been used as a promising therapeutic agent for cancer. Studies have shown that inhibition of PTGS2 is a potential therapeutic approach for colorectal cancer (CRC), however further exploration is needed [22]. PTGS2 is overexpressed in lung cancer and promotes tumor proliferation, invasion, angiogenesis and resistance to apoptosis. The traditional Chinese medicine scutellaria baicalensis oligonucleotide drug (HQi-sRNA-2), which targets PTGS2, significantly inhibited proliferation, migration and invasion, and induced apoptosis in the human lung cancer cell line NCI-H460 [23]. BCL2 and PTGS2 are abundant and associated with low patient survival in gastric cancer, and are closely related to cisplatin resistance. In a xenograft model, PTGS2 inhibition by celecoxib significantly enhanced the cytotoxic efficacy of cisplatin in cisplatin-resistant gastric cancer by suppressing the expression of PTGS2 and BCL2 regulated by ERK1/2 and P38 signaling axes, suggesting that PTGS2 may be used as an adjuvant therapeutic target for reversing chemoresistance in a subpopulation of cisplatin-resistant gastric cancers [24]. 5) Hypoxia-inducible factor 1 α (HIF1A) acts as a transcription factor (TF) that interacts with other cofactor TFs to activate transcriptional responses, such as increased glucose uptake and angiogenesis, to promote gene expression in hypoxic cancer cells. Since HIF1A-regulated responses promote cancer cell survival under hypoxic conditions, many studies have been transferred to HIF1A-targeted therapies. Although many attempts at HIF1A-targeted therapy have been proposed, one of the most intriguing prospects is targeting HIF1A through its protein-protein interaction (PPI). This approach has been shown to have high diagnostic potential. Also, like many TFs, HIF1A can interact with a variety of cofactor TFs to effectively control gene expression [25]. For example, HIF1A is known to dimerize with its binding chaperone, the aryl hydrocarbon receptor nuclear transport protein (ARNT), to synergistically regulate target genes. HIF1A positively regulates olfactin 4, leading to hypoxia-induced invasion, epithelial mesenchymal cell transformation, and chemoresistance in non-small cell lung cancer cells. In addition, HIF1A and HIF2A are critical for cisplatin resistance. In lung cancer cells, HIF1A and HIF2A induce cisplatin resistance by enhancing autophagy induction under hypoxic conditions. Overexpression of HIF1A and HIF2A also accelerates the excretion of chemotherapeutic agents from the tumor cells and induces aberrant intracellular DNA damage repair, which reduces the sensitivity of the cells to DNA-damage-based chemotherapeutic agents and leads to multidrug resistance in tumors. However, due to the complexity of the tumor cell microenvironment, studies on the mechanism of HIF-induced tumor drug resistance are still limited [26].6) TGFB1 as a member of the TGF-β superfamily. It can participate in the processes of cell proliferation, differentiation and growth. TGFB1 is synthesized by both healthy epithelial cells and tumor cells and plays a dual role in cancer. It can contribute to cancer development by suppressing immune surveillance, promoting epithelial-mesenchymal transition, and facilitating metastasis, or it can inhibit tumor growth by directly impeding cell cycle progression, leading to growth arrest and activation of apoptotic pathways. In addition, TGFB1 regulates and controls the expression and activation of other growth factors, including interferon-γ and tumor necrosis factor-α. Upon activation, TGFB1 binds to the ubiquitously expressed

cell-surface TGFB1 type I receptor (TGFBR1) and type II receptor (TGFBR2). These receptors are transmembrane serine/threonine kinases associated with tumorigenesis [27].

4.3 GO and KEGG enrichment analysis GO enrichment analysis showed that "SCH-AF" mainly exerts its anti-NSCLC effect through the regulation of inorganic substances, lipopolysaccharides, bacterial molecular responses, the influence of various transcriptional regulatory complexes, and the regulation of cytokine receptors, transcriptional co-regulators, etc. The results of KEGG pathway showed that the treatment process of "SCH-AF" against NSCLC involves several apoptosis and autophagy-related signaling pathways. The results of KEGG pathway showed that the therapeutic process of "SCH-AF" against NSCLC involves multiple apoptosis and autophagy-related signaling pathways. IL-17 signaling pathway: Interleukin 17 (IL-17) is a highly versatile pro-inflammatory cytokine with multiple roles in the host defense response to mucosal infection. infection; moreover, it is a major pathological cytokine and therapeutic target in many autoimmune, inflammatory diseases and cancers.IL-17-mediated signaling pathways are closely associated with tumorigenesis, proliferation, metastasis, and drug resistance under pathological conditions. In addition to autoimmunity, abnormal IL-17 levels are an important pathogenic factor in the early and late development of human cancers. Alterations in IL-17 levels have a significant impact on tumor development in a variety of organs, including the colon, such as the lung, liver, pancreas, and bile ducts. In addition, clinical studies have shown that high serum IL-17 levels are associated with poorer prognosis and resistance to radiotherapy in patients with a variety of solid tumors, suggesting that IL-17 inhibition may be effective in suppressing tumor metastasis and enhancing the sensitivity of cancer cells to radiotherapy. However, some researchers have suggested that IL-17 can act as both a promoter and an inhibitor of tumor progression in humans, and elucidating the mechanism of its inhibition in cancer may also be useful in developing future tumor therapies. Current research on IL-17 has focused on its important role in tumorigenesis, progression, and regression. Recent evidence suggests that chronic inflammation may be a predisposing risk factor for many tumors. Chronic IL-17-induced inflammatory responses are thought to be important in mediating cellular carcinogenesis, promoting tumor cell proliferation and metastasis, and inducing immune tolerance in cancer cells [28].2) TNF signaling pathway: Tumor necrosis factor-α (TNFα) is a pleiotropic pro-inflammatory cytokine of the TNF superfamily. In addition to its role in the immune response through the formation of complexes by ligand-activated TNFα receptors to activate various intracellular signaling pathways (e.g., MAPK, Akt, NF-κB, etc.), it plays a key role in regulating cell death and proliferation. TNFα tightly regulates the activity of key signaling proteins through phosphorylation or ubiquitination, ultimately generating specific cellular responses. Dysregulated TNFα signaling has been associated with inflammatory diseases, neurological disorders, and cancer. TNFα has been shown to exert opposing effects on cancer cells, and thus a detailed understanding of the phenomenon of TNFα signaling is essential to explore its pleiotropic role in malignancy and its potential as a drug target or anticancer therapy [29]. Tumor necrosis factor α (TNF-α) is the most potent cytokine with anti-tumor effects discovered to date. TNF-α enhances the sensitivity of lung cancer cells to radiotherapy and inhibits cancer metastasis through activation of the SAKP/JNK signaling pathway [30].

5. Conclusion

In this study, we investigated the mechanism of action of "SCH-AF" in the treatment of NSCLC using network pharmacology, and demonstrated that Ishimitsu-Precipitant acted on key targets such as MMP9, CASP3, BCL2, PTGS2, HIF1A, TGFB1, etc., mainly through the active ingredients such as quercetin, beta-sitosterol and other active ingredients. It has been demonstrated that the drug pair "SCH-AF", through various active ingredients such as quercetin, beta-sitosterol, etc., mainly acts on key targets such as MMP9, CASP3, BCL2, PTGS2, HIF1A, TGFB1, etc., and regulates various signaling pathways such as Pathways in cancer, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway and other signaling pathways. signaling pathway, TNF signaling pathway, HIF-1 signaling pathway and other signaling pathways, mainly through the regulation of cell proliferation and apoptosis, inhibition of invasion and metastasis and other aspects of the anti-NSCLC role. The mechanism of action of "SCH-AF" in the treatment of NSCLC is characterized by multi-components, multi-targets, and multi-pathways, which provides a theoretical basis for the clinical use of activating blood circulation and promoting qi, and resolving blood stasis and dispersing stagnation in the treatment of NSCLC. The limitations of the network pharmacology method are that it is mainly used for predictive analysis, while the clinical efficacy is related to various factors, such as drug dosage, dosage form and decoction time, and the conclusions obtained need to be further verified by experiments.

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References

[1] Bray F, Laversanne M, Sung H, et al.. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May-Jun;74(3):229-263.

[2] H.M. Abdelaziz, M. Gaber, M.M. Abd-Elwakil, et al.. Elzoghby Inhalable particulate drug delivery systems for lung cancer therapy: Nanoparticles, microparticles, nanocomposites and nanoaggregates J. Control Release, 269 (2018), pp. 374-392.

[3] LING C Q, YUE Q, CHEN L. Three advantages of using traditional Chinese medicine to prevent and treat tumor[J]. J Integr Med, 2014, 12(4):331-335.

[4] Yang Wenjing, Zhang Ganlin, Yang Guowang. Exploration of anti-tumor therapeutic mechanism by activating blood circulation and removing blood stasis [J]. Liaoning Journal of Chinese Medicine, 2019, 46(11):2311-2314.

[5] WANG Yanhong, GUAN Feng, WANG Ri. Progress of research on Chinese medicine Shihmianuan [J]. Chinese Pharmacy, 2006(24): 1909 -1910.
[6] ZHOU Li, LI Chen, CAO Fengjun, et al. Anti-cancer Chinese medicine Prevotella / Yan Cui: Herbal variation and resource distribution [J]. Journal of Chinese Medicine Clinics, 2019, 31(5):799 -803.
[7] Jiao Minghao, Qi Yuanfu. Introduction of Prof. Qi Yuanfu's experience in treating lung cancer by using medicinal pairs[J]. Yunnan Journal of Traditional Chinese Medicine, 2020, 41(11):9-12.
[8] N. Wathoni, L.E. Puluhulawa, I.M. Joni, et al.. Gozali Monoclonal antibody as a targeting mediator for nanoparticle targeted delivery system for lung cancer Drug Deliv., 29 (1) (2022), pp. 2959-2970
[9] L. E. MathieuLN, A.K. Sinha, P.S. Mishra-Kalyani, et al.. Singh FDA approval summary: atezolizumab as adjuvant treatment following surgical resection and platinum-based chemotherapy for stage II to IIIA NSCLC Clin. Cancer Res, 29 (16) (2023), pp. 2973-2978
[10] X. Zhu, Z. Yu, L. Feng, et al.. Zheng Chitosan-based nanoparticle co-delivery of docetaxel and curcumin ameliorates anti-tumor chemoimmunotherapy in lung cancer Carbohydr. Polym., 268 (2021), Article 118237
[11] Li Y, Yan B, He S. Advances and challenges in the treatment of lung cancer. Biomed Pharmacother. 2023 Dec 31;169:115891.
[12] You Jiafeng, Yu Mingwei, Shang Beibei, et al. Effect of Zilongjin tablets on survival time of patients with middle and advanced non-small cell lung cancer[J]. Beijing Traditional Chinese Medicine, 2024, 43(03):250-255.
[13] Lotfi N, Yousefi Z, Golabi M, et al.. The potential anti-cancer effects of quercetin on blood, prostate and lung cancers: An update. Front Immunol. 2023 Feb 28;14:1077531.
[14] Kashyap D, Garg VK, Tuli HS, et al.. Fisetin and quercetin: Promising flavonoids with chemopreventive potential. Biomolecules (2019) 9(5).
[15] Izdebska M, Hałas-Wiśniewska M, Zielińska W, et al.. Lidocaine induces protective autophagy in rat C6 glioma cell line. Int J Oncol (2019) 54(3):1099–111.
[16] Mukherjee A, Khuda-Bukhsh AR. Quercetin down-regulates IL-6/STAT-3 signals to induce mitochondrial-mediated apoptosis in a nonsmall- cell lung-cancer cell line, A549. J Pharmacopunct (2015) 18(1):19–26.
[17] Sacks D, Baxter B, Campbell BCV, et al.. Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. Int J Stroke (2018) 13(6):612–32.
[18] Chen Y, Yang Y, Wang N, et al.. β-Sitosterol suppresses hepatocellular carcinoma growth and metastasis via FOXM1-regulated Wnt/β-catenin pathway. J Cell Mol Med. 2024 Feb;28(3):e18072.
[19] Rashid ZA, Bardaweel SK. Novel Matrix Metalloproteinase-9 (MMP-9) Inhibitors in Cancer Treatment. Int J Mol Sci. 2023 Jul 28;24(15):12133.
[20] Bhat AA, Thapa R, Afzal O, et al.. The pyroptotic role of Caspase-3/GSDME signalling pathway among various cancer: A Review. Int J Biol Macromol. 2023 Jul 1;242(Pt 2):124832.
[21] Kaloni D, Diepstraten ST, Strasser A, et al.. BCL-2 protein family: attractive targets for cancer therapy. Apoptosis. 2023 Feb;28(1-2):20-38.
[22] Zheng W, Guo Y, Kahar A, et al.. RUNX1-induced upregulation of PTGS2 enhances cell growth, migration and invasion in colorectal cancer cells. Sci Rep. 2024 May 22;14(1):11670.
[23] Lin Y, Sun N, Liu D, et al.. COX-2/PTGS2-targeted herbal-derived oligonucleotide drug HQi-sRNA-2 was effective in spontaneous mouse lung cancer model. IUBMB Life. 2024 Jul 25.
[24] Lin XM, Li S, Zhou C, et al.. Cisplatin induces chemoresistance through the PTGS2-mediated anti-apoptosis in gastric cancer. Int J Biochem Cell Biol. 2019 Nov;116:105610.
[25] Zhang Y, Wang S, Hu H, et al.. A systematic study of HIF1A cofactors in hypoxic cancer cells. Sci Rep. 2022 Nov 8;12(1):18962.
[26] Wu Q, You L, Nepovimova E, et al.. Hypoxia-inducible factors: master regulators of hypoxic tumor immune escape. J Hematol Oncol. 2022 Jun 3;15(1):77.
[27] Chen Z, Ding C, Chen J, et al.. Pan-cancer analysis revealing the multidimensional expression and prognostic and immunologic roles of TGFB1 in cancer. J Int Med Res. 2024 Jan;52(1):3000605231221361.
[28] Huangfu L, Li R, Huang Y, et al.. The IL-17 family in diseases: from bench to bedside. Signal Transduct Target Ther. 2023 Oct 11;8(1):402.
[29] Manohar SM. At the Crossroads of TNF α Signaling and Cancer. Curr Mol Pharmacol. 2024; 17(1): e060923220758.
[30] Zhang Z, Ma J, Li S, et al.. Integrating Network Pharmacology to Explore the Pharmacology Mechanisms of Meliae Cortex Against Hepatocellular Carcinoma [J]. Journal of Contemporary Medical Practice, 2024, 6(6):135-140.