Exploring the molecular mechanism of Baoyuan decoction in the treatment of lung cancer based on network pharmacology and molecular docking

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Author contributions

Ri-Cheng Jiang, Hao Jin and Dan Wang designed this study; Dan Wang and Zhen-Hua Ge screened and extracted the data; Dan Wang, Zhen-Hua Ge, and Bo-Ya Guan conducted and visualized the data analysis; Dan Wang wrote this manuscript. Ri-Cheng Jiang, Hao Jin, Zhen-Hua Ge and Bo-Ya Guan reviewed the manuscript’s intellectual content. Dan Wang is the first author, Zhen-Hua Ge is the second author and Bo-Ya Guan is the third author of this study. Ri-Cheng Jiang, Hao Jin are the corresponding authors, and Ri-Cheng Jiang is the primary corresponding author. All authors discussed, edited, and approved the final version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

TCM, traditional Chinese medicine; TCMSP, Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform; PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL-6, interleukin-6; EGFR, epithelial growth factor receptor; STAT3, signal transducer and activator of transcription 3; EMT, epithelial-to-mesenchymal transition; NSCLC, non-small cell lung cancer; TNF, tumor necrosis factor; BP, biological processes; MF, molecular functions; CC, cellular components.

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Abstract

Background: Baoyuan decoction is used clinically as an adjuvant treatment for lung cancer. However, the underlying mechanism remains unclear. Therefore, this study aimed to explore the mechanism of action of Baoyuan decoction in lung cancer treatment using network pharmacology and molecular docking technology. Methods: The Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform and SwissTargetPrediction databases were used to screen the active ingredients of Baoyuan decoction and their relevant targets. Lung cancer-related targets were obtained from the GeneCards, Online Mendelian Inheritance in Man, and DrugBank databases. Protein-protein interaction network of the common targets was constructed using the STRING database and analyzed using Cytoscape software 3.10.1. Furthermore, Gene Ontology enrichment, Kyoto Encyclopedia of Genes and Genomes pathway analyses and visualization of common genes were performed using the R software. Finally, molecular docking of the selected key ingredients and targets was performed, and the results were verified using AutoDock Vina software. Results: We identified 142 potential active ingredients, 3624 potential lung cancer-related targets, and 341 common drug targets. A total of 72 core targets were identified, of which AKT1, TP53, interleukin-6, epithelial growth factor receptor, and signal transducer and activator of transcription 3 were key. A total of 4116 items were obtained via Gene Ontology enrichment analyses. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses revealed 189 related signaling pathways, including the PI3K-Akt, AGE-RAGE signaling pathways in diabetic complications, FOXO, and TH signaling pathways, which are involved in cell proliferation, autophagy, metastasis, invasion, radiation resistance, and chemotherapy resistance in the lung cancer microenvironment. The molecular docking results suggested that the key ingredients had a strong affinity for key targets. Conclusion: This study demonstrates that Baoyuan decoction plays a key therapeutic role in a complex manner involving multiple ingredients, targets, and pathways in lung cancer. Our findings are anticipated to provide new ideas for follow-up experimental research and clinical application.

Keywords: Baoyuan decoction; lung cancer; network pharmacology; molecular docking; mechanism research
Background

Lung cancer is a common cause of cancer-related morbidity and mortality worldwide [1]. According to data released by the National Cancer Center in 2023, lung cancer had the highest number of new cases and deaths from malignant tumors in China in 2016 [2]. Despite various clinical treatments for lung cancer, such as surgery, chemoradiotherapy, targeted therapy, and immunotherapy, the five-year survival rate for patients remains less than 15% owing to late detection, postoperative complications, adverse reactions, and drug resistance [3, 4]; these side effects severely impact the quality of life of patients. In recent years, numerous high-level evidence-based medical studies have explored lung cancer treatment with traditional Chinese medicine (TCM). The integration of TCM and Western medicine in the treatment of lung cancer has improved the efficacy of comprehensive treatment. Various explorations and practices have been undertaken in China to diagnose and treat lung cancer using this integrated approach [5]. TCM has clinical significance in the maintenance treatment of lung cancer, reducing clinical symptoms and drug side effects, improving quality of life, and delaying disease progression [6, 7]. Baoyuan decoction, listed in the Catalogue of Ancient Classic Prescriptions (First Batch), was first described in Zhihong Sun’s Concise Medical Formula during the Ming Dynasty. This classic prescription of TCM is used to replenish vital energy and consists of Ginseng Radix, Astragali Radix, Cinnamomi Cortex, Glycyrrhizae Radix, and Zingiberis Rhizoma, which benefits Qi (Qi refers to the basic substance that constitutes the human body and maintains life activities, and is the unity of substance and function) and Yang (in Chinese philosophy, the masculine, active and positive principle, characterized by light, warmth, dryness, activity, etc.). In recent years, Baoyuan decoction has often been used in the treatment of cardiovascular, hematological, central nervous, and respiratory system diseases, lung cancer, and infectious diseases [8]. A few clinical or preclinical studies have tried to elucidate the molecular mechanism of Baoyuan decoction in the treatment of lung cancer. The underlying mechanism remains unclear; however, existing studies have demonstrated the potential anticancer effects of its components in other types of cancer. Similarly, there have been studies demonstrating the efficacy and mechanisms of multiple herbal formulations in non-small cell lung cancers (NSCLCs).

Network pharmacology is an emerging discipline that takes a holistic and systematic approach to designing multi-target drug molecules through an interdisciplinary application and the analysis and reintegration of the overall biological network. Network visualization is used to analyze the complex interactions among diseases, drugs, and targets, and to interpret and evaluate the synergistic mechanism between the active ingredients of TCM. Molecular docking technology is used to verify the binding ability of the screened active ingredients to key core targets. In this study, the network pharmacology method was used to explore the mechanism of action of Baoyuan decoction in treating lung cancer, providing new ideas for the precise clinical treatment of this disease. Figure 1 illustrates the protocols used in this study.

Figure 1 Potential mechanism of Baoyuan decoction in the treatment of lung cancer. TCMSP, Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
Screening of effective active ingredients of Baoyuan decoction and construction of active ingredients-targets network

The Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php/)[9] was used to identify the main chemical constituents of Ginseng Radix, Astragal Radix, Cinnamomi Cortex, Glycyrrhiza Radix, and Zingiberis Rhizoma. Owing to the small number of active ingredients that met this condition, Cinnamomi Cortex was screened for oral bioavailability ≥ 30% and drug-likeness ≥ 0.1, while the other herbs were screened for oral bioavailability ≥ 30% and drug-likeness ≥ 0.18. The chemical components for which no target information was retrieved were screened with a score of “high” in gastrointestinal absorption and at least 2 "Yes" in druglike-ness. Canonical SMILES data were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [10], and imported into the SwissTargetPrediction database (http://www.swistargetprediction.ch/) [11], with the species was selected as “Homo sapiens” for target prediction. Baoyuan decoction-related targets with a probability greater than 0 were predicted. All drug targets were aggregated and normalized using the UniProt database (https://www.uniprot.org/) [12], with duplicate values removed individually based on the ingredients. Cytoscape 3.10.1 software was used to construct the network of “Baoyuan decoction-active ingredient targets,” and topological analysis and visualization were conducted to determine the main active ingredients of Baoyuan decoction involved in treating lung cancer.

Identification of lung cancer-related targets

We searched for “lung cancer” as the keyword and the targets greater than or equal to the median of 9.95 were screened based on the relevance score in the GeneCards database (https://www.genecards.org/) [13], the Online Mendelian Inheritance in Man database (https://omim.org/) [14], and the DrugBank database (https://go.drugbank.com/) [15]. The targets screened from the three databases were summarized, and duplicate values were removed to obtain all lung cancer targets.

Construction of protein-protein interaction (PPI) network and identification of core targets

The active ingredients-related targets of Baoyuan decoction and the disease targets of lung cancer were imported into Venn 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html), and a Venn diagram was plotted to identify the intersecting targets, which were considered potential therapeutic targets of Baoyuan decoction in the treatment of lung cancer. The intersecting targets were uploaded to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) 12.0 database (https://string-db.org/) [16]. And the PPI network of potential therapeutic targets was constructed with the following conditions: the species was “Homo sapiens” and the reliability was “medium confidence ≥ 0.4.” Subsequently, the PPI network information in tab-separated values format was imported into the Cytoscape 3.10.1 software, in which the topology algorithm of the network was performed using the “CentiScaPe 2.2 Menu” tool to obtain topology parameters of nodes. Core targets were screened based on the closeness, betweenness, and degree values greater than the median.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The clusterProfiler package of R software (version 4.3.2) was used to analyze and visualize the GO function and KEGG pathway enrichment analysis of potential therapeutic targets. The background database and gene set species were set as “Homo sapiens,” and the applicable threshold was set to an adjusted P-value < 0.05. In addition, the results are represented separately using a bubble plot. The network of active ingredients, potential targets, and signaling pathways was visualized using Cytoscape 3.10.1 software.

Verification with molecular docking

Molecular docking was used to analyze the relationship between the key components of Baoyuan decoction and the core targets of lung cancer. The 2D structures of the small-molecule ligands of the main active ingredients of Baoyuan decoction were downloaded from the PubChem database, and the 3D structures of the protein receptors of the core targets were downloaded from the Protein Data Bank database (https://www.rcsb.org/) [17]. Subsequently, these structures were imported into PyMOL (version 4.3.0) software (https:// pymol.org/) to separate the original ligand and protein structure, dehydrate, and remove organic matter. AutoDock Tool 1.5.6 software was used for hydrogenation and charge calculation. The atom type was specified as AD4 and the protein structure format and small-molecule ligand were converted from pdb to pdbqt. Finally, the docking activities between the target and ligand were calculated using AutoDock Vina (version 1.1.2). Six docking combinations with representative binding energies were visualized using PyMOL and Discovery Studio software.

Statistical analysis

Statistical analyses were automatically conducted for molecular docking and network pharmacology utilizing bioinformatic tools available through the software and platforms mentioned above. A P-value < 0.05 denoted statistical significance in the enrichment of GO functional and KEGG pathway analyses.

Results

Main active ingredients and related targets of Baoyuan decoction

We deleted the active ingredients and duplicates that did not have corresponding targets after screening the TCMSP database and obtained 142 active ingredients, including 19 Ginseng Radix, 19 Astragal Radix, 9 Cinnamomi Cortex, 5 Zingiberis Rhizoma, and 90 Glycyrrhizae Radix (Supplementary Table S1). After merging and removing duplicate targets from the targets screened in the TCMSP database and the predicted targets in the SwissTargetPrediction database, 648 targets were obtained after standard conversion processing by the UniProt database. The active ingredient-target network of Baoyuan decoction was constructed using Cytoscape 3.10.1 software, as shown in Figure 2. Eleven main active ingredients with degree values greater than 70 were screened (Supplementary Table S2), among which A1–A6 were the common components of Astragali Radix and Glycyrrhizae Radix, B1 and B2 were the common components of Ginseng Radix and Zingiberis Rhizoma, and C1 was the common component of Astragali Radix, Glycyrrhizae Radix, and Ginseng Radix.

Potential therapeutic targets of Baoyuan decoction used in the treatment of lung cancer

Lung cancer-related targets were selected from the GeneCards, Online Mendelian Inheritance in Man, and DrugBank databases using the search term “lung cancer,” with 3624 targets identified after deleting duplicate targets. There were 341 intersecting targets between active ingredient-related targets and lung cancer-related targets, which were considered potential therapeutic targets of Baoyuan decoction used in lung cancer treatment, as depicted in the Venn diagram (Figure 3).

PPI network visualization and analysis

The STRING database was used to analyze the relationship between the 341 potential therapeutic targets. The “CentiScaPe 2.2 Menu” tool of Cytoscape 3.10.1 software was used for the analysis and visualization of the PPI network, which included a total of 785 nodes and 3563 edges, with an average degree value of 65.2 and an average local clustering coefficient of 0.596. The greater the degree, the more significant the role of the protein in the network. Based on the three parameters (closeness, betweenness, and degree), 72 core targets were obtained from the potential therapeutic targets, as illustrated in Figure...
Among these, the top six targets with the highest correlation were AKT1, TP53, tumor necrosis factor (TNF), interleukin-6 (IL-6), epithelial growth factor receptor (EGFR), and signal transducer and activator of transcription 3 (STAT3) (Table 1), suggesting that these targets play a key role in the PPI network and may be the key targets which significantly influence the effects of Baoyuan decoction in the treatment of lung cancer.

**GO enrichment analysis**

GO enrichment analysis of 341 potential therapeutic targets was performed using R software to determine the biological mechanisms of Baoyuan decoction in the treatment of lung cancer. The analysis identified 4116 items with significance, including 3551 biological processes (BP), 143 cellular components (CC), and 422 molecular functions (MF) associated with the targets. The top 10 GO terms were selected for visualization in a bubble plot according to the number of genes (Figure 5). Bubble color and size indicate the adjusted P-value and enriched gene, respectively. The main BP terms included positive regulation of kinase activity and responses to oxidative stress, xenobiotic stimulus, molecules of bacterial origin, and lipopolysaccharide. The main enriched CC terms were membrane raft, membrane microdomain, transcription regulator complex, external side of plasma membrane, and transferase complex transferring. The most enriched MF terms were DNA binding, RNA binding, transcription factor binding, and protein kinase activity. Core targets such as protein kinases and transcriptases are involved in gene transcription, as supported by the MF results. These results suggest that Baoyuan decoction may be effective for the treatment of lung cancer by participating in and regulating these items.
Figure 4 PPI network of core targets. Larger circles and darker coloured targets indicate core targets. PPI, protein-protein interaction.

Table 1 Topology analysis of the key targets of the PPI network

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>Protein target</th>
<th>Closeness unDir</th>
<th>Betweenness unDir</th>
<th>Degree unDir</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AKT1</td>
<td>RAC-alpha serine/threonine-protein kinase</td>
<td>0.002325581</td>
<td>4376.106981</td>
<td>247</td>
<td>247</td>
</tr>
<tr>
<td>2</td>
<td>TP53</td>
<td>Tumor protein p53</td>
<td>0.002298851</td>
<td>4644.828886</td>
<td>242</td>
<td>242</td>
</tr>
<tr>
<td>3</td>
<td>TNF</td>
<td>Tumor necrosis factor</td>
<td>0.002202643</td>
<td>3071.646046</td>
<td>222</td>
<td>222</td>
</tr>
<tr>
<td>4</td>
<td>IL-6</td>
<td>Interleukin-6</td>
<td>0.002178649</td>
<td>2645.43336</td>
<td>217</td>
<td>217</td>
</tr>
<tr>
<td>5</td>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
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<td>3239.925306</td>
<td>211</td>
<td>211</td>
</tr>
<tr>
<td>6</td>
<td>STAT3</td>
<td>Signal transducer and activator of transcription 3</td>
<td>0.002132196</td>
<td>2079.580375</td>
<td>210</td>
<td>210</td>
</tr>
</tbody>
</table>

PPI, protein-protein interaction.

Figure 5 Bubble plot of the top 10 BP, MF, and CC categories associated with Baoyuan decoction and lung cancer. The ordinate represents the GO function terms; the abscissa represents the number of genes enriched on the GO entry as a proportion of the common targets. BP, biological processes; MF, molecular functions; CC, cellular components; GO, Gene Ontology.

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**KEGG pathway enrichment analysis**
The metabolic pathways of potential therapeutic targets for the treatment of lung cancer were identified using KEGG pathway enrichment analyses. A total of 189 signaling pathways were obtained using the R software. The top 20 signaling pathways included infection, tumor, endocrine, and other signaling pathways. We plotted the top 20 signaling pathways in a bubble plot (Figure 6), sorted by their P-values from smallest to largest. The results revealed that the core targets were mainly enriched in the PI3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complications, FOXO signaling pathway, and TH signaling pathway. To further analyze the relationship between the active ingredients, core targets, and key pathways, a network of active compound-potential targets-signaling pathways was constructed using Cytoscape v3.10.1, which comprised 222 nodes and 1505 edges (Figure 7). Eleven main active ingredients exhibited strong correlations with key targets. Therefore, these main active ingredients were selected for molecular docking using the Auto Dock Vina software with the key targets, including AKT1, TP53, TNF, IL-6, EGFR, and STAT3.

**Figure 6** KEGG pathway enrichment analysis of potential therapeutic targets. KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Figure 7** Visualization of active ingredients-core targets-signaling pathways. Each of the yellow diamond nodes in the middle represents the potential targets corresponding to the TCM ingredients. The dark blue circle nodes represent the herbal names of the Baoyuan decoction, the hexagons of different color nodes represent the active ingredients of herbs, and the octagons of different color nodes represent the common targets. TCM, traditional Chinese medicine.
Molecular docking
The 11 main active ingredients of Baoyuan decoction were selected as candidate docking active ingredients, and AKT1, TP53, TNF, IL-6, EGFR, and STAT3 were selected as candidate docking targets. The binding energy is inversely correlated with the affinity between the ligand and receptor. If the binding energy of the ligand to the target protein is \( \leq -5 \text{ kcal/mol} \), the ligand and receptor protein have good binding ability, and the structural type has a strong docking activity at the binding energy of \( \leq -7 \text{ kcal/mol} \). A total of 66 data points were obtained using the binding energy value, and GraphPad Prism 8.0.2 software was used to create a heat map (Figure 8). A total of 54 data points had binding energies less than \(-8.5 \text{ kcal/mol}\), accounting for 81.82%; eight points had resultant energy of less than \(-9 \text{ kcal/mol}\), accounting for 12.12%, and four points had a capacity less than \(-10 \text{ kcal/mol}\), accounting for 6.06%. These results can be used as a basis for the screening of Baoyuan decoction in the treatment of lung cancer. Six combinations with better binding energies were selected for visualization, as shown in Figure 9.

**Figure 8** Heatmap of molecular docking binding energy of Baoyuan decoction with corresponding key targets, unit (kcal/mol). TP53, tumor protein 53; TNF, tumor necrosis factor; IL-6, interleukin-6; EGFR, epithelial growth factor receptor; STAT3, signal transducer and activator of transcription.

**Figure 9** Visualization of molecular docking between active ingredients of Baoyuan decoction and key targets. The molecular docking models are as follows: (a) Celabenzine with EGFR. (b) Formononetin with TNF. (c) Isoflavonone with TNF. (d) Isorhamnetin with STAT3. (e) Quercetin with EGFR. (f) Celabenzine with STAT3. TNF, tumor necrosis factor; EGFR, epithelial growth factor receptor; STAT3, signal transducer and activator of transcription.
Discussion

Lung cancer belongs to the categories of “lung accumulation” and “respiration,” and the diseases are collectively referred to as “lung cancer disease” in TCM, which believes that the overall pathogenesis of lung cancer is due to deficiency and reality. The primary pathogenesis of lung cancer is a combination of Qi, blood, phlegm, stasis, and poison, and its clinical manifestations are complex and diverse. Approximately 85% of lung cancers are NSCLC [18], while the remainder are small cell lung cancers. Baoyuan decoction is a representative meridian formula for tonifying vital energy. The formula increases vital energy, strengthens the spleen, protects the lungs, and warms the kidneys; thereby enhancing the patient’s immunity and improving comprehensive therapeutic efficacy, which can be used for treating vital energy weakness. Clinically, the Baoyuan decoction has a therapeutic effect on lung cancer, there have been studies demonstrating the efficacy and mechanisms of multiple herbal formulations in NSCLC. However, its precise mechanism of action remains unclear.

In the present study, we used network pharmacology to predict the mechanism underlying the therapeutic effects of Baoyuan decoction in the treatment of lung cancer. We identified and screened 11 active ingredients of Baoyuan decoction. Quercetin, kaempferol, and isoflavanone serve as the basis for anti-lung cancer activity.

Flavonoids, such as quercetin [19] and kaempferol [20], have been recently found to exert strong anticancer effects on various cancers. Modern pharmacological studies have confirmed that quercetin has a good preventive effect against lung cancer [21]. Quercetin and its derivatives arrest the G0/G1 phase of the cancer cell cycle, activate the PI3K/Akt molecular pathway, reduce proliferation, and induce apoptosis in cancer cells by inhibiting the synthesis of proteins essential for mitosis [22]. Quercetin promotes the induction of apoptosis in NSCLC cells by downregulating IL-6/STAT3 signaling expression, inhibits the epithelial-to-mesenchymal transition (EMT) process in lung cancer cells by altering the AKT/MAPK/β-catenin signaling pathway and inhibiting AKT-mediated β-catenin nuclear translocation [23], and may exert a strong cytotoxic effect on NSCLC cells harboring the EGFR C797S mutation by inhibiting the expression of tyrosine kinase receptor AXL and inducing apoptosis [24]. Furthermore, when quercetin is combined with commonly used anticancer drugs in clinical practice, drug resistance can be reversed and the efficacy of lung cancer treatment can be enhanced [25, 26].

Kaempferol affects the growth of NSCLC cells by regulating the PI3K/Akt signaling pathway [20]. Fouzder et al. demonstrated that kaempferol induces reactive oxygen species production and apoptosis in NSCLC cells, and enhances the efficacy of chemotherapy by downregulating the expression of Nrf2 and its downstream-related proteins and inhibiting the Nrf2 pathway [27]. In addition, kaempferol enhances the effect of radiotherapy in lung cancer by inhibiting the PI3K/Akt and ERK pathways and activating the mitochondrial apoptosis pathway.

As an intermediate metabolite of quercetin, isorhamnetin selectively blocks the Akt/ERK1/2 signaling pathway to inhibit EMT and the invasion and metastasis of lung cancer cells [28]; isorhamnetin also enhances the radiosensitivity of A549 cells through IL-13 and NF-κB signaling pathways [29] and exerts anti-lung cancer effects by partially modulating the p53 signaling pathway.

Formononetin inhibits NSCLC tumor growth by inhibiting the EGFR-Akt-Mcl-1 axis in NSCLC [30]. Li et al. found that formononetin may affect the cell cycle by downregulating the expression of cyclin E1 and promoting apoptosis by regulating the expression of Bcl-2 and Bax, thereby inhibiting the proliferation of NSCLC cells by upregulating the phosphorylation of P53 at Ser15/20 and enhancing its transcriptional activity in a dose-dependent manner. Formononetin promotes the cleavage of Caspase-3 and the expression of Bax. It effectively inhibits the activation of the PI3K/Akt signaling pathway in A549 cells. β-sitosterol inhibits cell proliferation by blocking the G2/M phase of A549 cells in a dose-dependent manner and promotes the apoptosis of lung cancer cells by upregulating the expression of p53 and Bax genes. Moreover, β-sitosterol induces G0/G1 cell cycle arrest and inhibits cell proliferation in A549 cells when combined with BAMBI overexpression [31]. MDM2/4 is the key negative regulator of p53 and has received increasing attention for its role in the occurrence and development of NSCLC.

Xing et al. reported that β-sitosterol and celabenzine in ginseng have a strong affinity for MDM2/4 protein, and can regulate the P53 level of NSCLC cells by regulating MDM2/4 protein [32]. However, studies on celabenzine and lung cancer have not yet been reported. The same is true for isoflavonones and 3,22-dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxy-carbonyl-29-oic acid.

PPI network analysis identified AKT1, TP53, TNF, IL-6, EGFR, and STAT3 as key targets of Baoyuan decoction in the treatment of lung cancer. AKT1 is a member of the AKT kinase family, which is involved in various BPs, such as cell proliferation, apoptosis, and metabolism, and its aberrant expression is associated with poor prognosis in cancer patients [33]. AKT1 is a key mediator of the PI3K/Akt signaling pathway, promoting the invasion and metastasis of KRAS or EGFR-mutated NSCLC cells, and increasing their sensitivity to chemotherapeutic agents. Therefore, AKT1 may play essential roles in the development and progression of NSCLC [34].

TP53 mutations are present in 86% of small cell lung cancers [35]. In advanced NSCLCs with EGFR mutations, approximately 50–60% have combined TP53 mutations [36, 37]. TP53 mutations can affect the efficacy of TKIs. Tan et al. found that a combination of TP53 mutations significantly reduced the median progression-free survival of patients treated with TKIs, regardless of the type of EGFR mutation they harbored [38]. Alterations in TP53 expression are associated with lower drug resistance and shorter survival in patients with EGFR-mutant lung cancer [39].

TNF-α is an effective paracrine and autocrine mediator of inflammation and immune response, which can directly or indirectly induce STAT3 activation [40], metastasize and activate EGFR, and promote tumorigenesis and development. EGFR-mutated NSCLC highly expressed TNF-α in a mouse lung adenocarcinoma tissue model [41]. Biological effects of TNF-mediated adaptive feedback promote the survival and proliferation of NSCLC tumor cells [42]. It has been found that elevated serum TNF-α levels could stimulate the secretion of IL-6 by macrophages, and IL-6 is bound to its receptor to act on vascular endothelial growth factor, promoting the occurrence and development of lung cancer by enhancing the formation of tumor tissue microvessels. TNF-α and IL-6 promote the EMT process in NSCLC cells. In addition, multivariate regression analysis suggested that IL-6 levels in malignant pleural effusions might be an independent predictor of prognosis in EGFR-sensitive mutant NSCLC. Cancer-associated fibroblasts in lung cancer enhance the metastatic potential of lung cancer cells by activating the JAK2/STAT3 signaling pathway via IL-6 secretion. Decreased IL-6 levels in patients with NSCLC treated with PD-1 suppression are associated with improved progression-free survival [43]. IL-6 may inhibit STING-TBK1 signaling by activating the STAT3-AKT pathway and attenuating the killing effect of T-cells on NSCLC cells. Moreover, a study found that low pre-treatment of IL-6 levels in patients with NSCLC treated with PD-1 inhibitors were associated with clinical benefits, suggesting that IL-6 levels may be used as a predictor of the efficacy of immunotherapy.

EGFR driver mutations are the most common mutations in NSCLC, which lead to uncontrolled tumor proliferation by inducing the constitutive activation of epidermal growth factor tyrosine kinase [44] and are often used as targets for targeted therapy. EGFR induces STAT3 activation [45]. Additionally, by activating the MAPK signaling pathway [46, 47] and PI3K-Akt signaling pathways, EGFR inhibits tumor cell apoptosis and promotes tumor cell proliferation, angiogenesis, and metastasis, thereby participating in lung cancer progression.

The expression level of STAT3 significantly increased in lung cancer, and its abnormal activation is common in patients with lung adenocarcinoma. Patients with lung cancer and high STAT3
expression tend to present advanced tumor stages and experience lower three-year overall survival rate. Grabner et al. found that the downregulation of STAT3 expression promotes the formation of KRAS-mutated lung adenocarcinoma. Additionally, STAT3 is highly expressed in Afatinib-resistant 7790M-mutated NSCLC cells and may be involved in the development of acquired resistance mechanisms. STAT3 knockdown in a genetically engineered mouse model carrying human metastatic lung adenocarcinoma tissue inhibits tumor metastasis [48].

These potential targets involve a variety of BPs, such as kinase activity regulation, apoptosis, autophagy, EGFR-TKI, and chemoradiotherapy resistance, involving various elements, such as transcription factors, cytokines, driver genes, miRNAs, and other proteins, suggesting that Baoyuan decoction can be used in the treatment of lung cancer.

In this study, GO enrichment analysis provided an in-depth definition and description of the functions of potential therapeutic targets, revealing the potential mechanism of Baoyuan decoction in the treatment of lung cancer. Some important BP categories, such as the positive regulation of kinase activity and response to oxidative stress, are associated with these potential therapeutic targets. Moreover, the inflammatory response is closely related to the occurrence and progression of lung cancer, and several of the key targets screened in this study, including cytokines (such as TNF and IL-6), are involved in regulating the inflammatory microenvironment of lung cancer. The results of GO enrichment analysis indicates that Baoyuan decoction mainly affects the MFs of gene transcriptional regulation, phosphorylation of protein serine and threonine residues, and transmembrane receptor proteins in the treatment of lung cancer by participating in BPs such as the positive regulation of kinase activity, oxidative stress, and cellular response to chemical stimuli.

Multiple signaling pathways involved in the anti-lung cancer mechanism of Baoyuan decoction were identified in the KEGG enrichment analysis. The core gene targets were mainly enriched in the PI3K-AKT and AGE-RAGE signaling pathways in diabetic complications, FOXO, and TH signaling pathways. By regulating these signaling pathways, the BPs in which the core targets are involved can be mediated, indicating that these are signaling pathways closely related to the development and metastasis of lung cancer. Furthermore, these signaling pathways are closely related to the development and metastasis of lung cancer. Therefore, Baoyuan decoction may play an anti-lung cancer role by interfering with tumor cell proliferation, apoptosis, autophagy, metastasis, invasion, and chemoradiotherapy resistance. The PI3K/Akt signaling pathway plays a key role in the occurrence and development of lung cancer. This pathway and its related target proteins inhibit cell proliferation, induce apoptosis, promote autophagy [49], inhibit invasion and metastasis, and regulate immunity by regulating the expression of cyclins (e.g., cyclin D1) and apoptosis-related proteins (e.g., Bcl-2) in lung cancer. Moreover, the PI3K/Akt signaling pathway is closely related to targeted drug resistance [50], and the aberrant activation of the PI3K/Akt/mTOR pathway is one of the mechanisms by which patients with lung adenocarcinoma harboring EGFR-activating mutations acquire resistance to EGFR-TKI inhibitors. The main AGE receptor is RAGE, which is highly expressed in normal lung tissue and strongly adheres to the extracellular matrix and the surrounding matrix, suggesting that it may maintain the stability of the intrapulmonary environment. Reduced RAGE expression in NSCLC cells and decreased adhesion of lung histiocytes leads to increased tumor cell infiltration and metastasis [51]. It has been found that the AGE-RAGE signaling pathway may promote cell proliferation, migration, invasion, and EMT by activating the PI3K/Akt signaling pathway.

FOXOs are known tumor suppressors that promote apoptosis and inhibit cell proliferation; most FOXOs in tumors are in the cytoplasm in an inactive phosphorylated state to maintain normal cell proliferation and survival. The FOXO signaling pathway downstream of the PI3K/Akt signaling pathway. The activation of the PI3K/Akt pathway decreases FOXO activity and expression, AKT promotes phosphorylation of FOXO and inhibits its transcriptional function, which may lead to apoptosis. Apoptosis is involved in the BPs of sensitization to radiotherapy and tumor growth inhibition in NSCLC [52]. The anti-lung cancer effects of the FOXO signaling pathway are mainly reflected in the involvement of apoptosis, autophagy, invasion and metastasis, cell cycle, and regulation of resistance to anticancer drugs.

TH promotes tumor cell proliferation, anticancer cell apoptosis, and tumor-associated angiogenesis [53]. Patients with lung cancer exhibit significant alterations in the function of the hypothalamic-pituitary-thyroid axis and serum TH levels. Mousa et al. found that TH inhibited tumor metastasis by downregulating the expression of MMP, EGFR, and ERBB2 [54]. TH levels are sensitive and specific markers for clinical staging and tumor metastasis in patients with NSCLC. Despite these findings, this study had some limitations. First, this study mainly relied on computational analyses of network pharmacology and molecular docking techniques, which provided a theoretical basis and initial insights into the underlying mechanisms. Therefore, the results need to be confirmed by further experimental validation to confirm their reliability. Second, there may have been incomplete or inaccurate information in the literature and databases, which may have affected the results of our analyses. Therefore, future studies should combine cellular experiments and animal models to verify the effects of Baoyuan decoction on lung cancer treatment and further explore its specific molecular mechanisms.

**Conclusion**

Our research reveals that lung cancer treatment with Baoyuan decoction is a complex process involving multiple components, targets and pathways, based on network pharmacology and molecular docking. Baoyuan decoction regulates key targets, including AKT1, TP53, TNF-IL-6, EGFR, and STAT3, which are involved in the proliferation, metastasis, invasion, and chemoradiotherapy resistance of lung cancer cells, through the PI3K-Akt, AGE-RAGE, FOXO, and TH signaling pathways. This study provides new ideas and a theoretical basis for the clinical application of lung cancer treatment and development of new tumour-resistant drugs.

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