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Exploring the Mechanism of Comfrey in Lung Cancer Based on Network Pharmacology and Molecular Docking

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Abstract **Original Research Article**

Network pharmacology was used to investigate the active ingredients and mechanism of action of the Chinese medicine comfrey in lung cancer. Comfrey constituents and their targets were collected by searching the TCMSP and PubChem databases. The Gene Cards database was searched to extract target information for lung cancer. Based on the STRING database, Cytoscape 3.9.1 software was used to construct the PPI network of common targets of comfrey in lung cancer. We searched for common targets and performed GO and KEGG pathway enrichment analyses to build a 'network diagram' of TCM active ingredient-disease targets. The results of the pharmacological network analysis showed that after the screening, 12 compounds and 904 drug targets were obtained, and 147 drug-disease common targets were obtained, with AKT1, EGFR, TNF, and CASP3 as the core targets. After enrichment analysis of the above key targets, it was found that cancer pathways, prostate cancer, PI3K-Akt signaling pathway, endocrine resistance, and cancer proteoglycans were involved in the treatment of comfrey. The molecular docking results also showed that the comfrey compounds had good binding activities with the key targets, proving the accuracy of the network pharmacological prediction results. The results of this experiment provisionally verified the basic pharmacological effects of comfrey and its mechanism of action against lung cancer and provided directions for further research.

Keywords: Lung Cancer, Network pharmacology, Molecular docking, Comfrey, Mechanism of action.

1 INTRODUCTION

Lung cancer is a malignant tumour that originates in the trachea, bronchi, and lungs. Lung cancer is of bronchial origin and includes several major types: squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and large cell carcinoma. Surgery rates for lung cancer show a fluctuating downward trend, while chemotherapy rates are increasing. 5-year relative survival rates increase with age but remain low [1]. Lung cancer is the most common cancer and the leading cause of malignant death worldwide, killing approximately 1.76 million people each year, while there are approximately 2 million new cases each year and this number is increasing [2]. Despite various therapeutic approaches, such as early surgical resection combined with radiotherapy techniques, immunotherapy, molecular targeting, and biotherapy, the prognosis is inferior, especially the resistance and severe side effects caused by surgical treatment and radiotherapy are still difficult to eliminate [3]. In this context, traditional Chinese medicine is considered a potential therapeutic agent in treating lung cancer and other diseases with its unique advantages of multi-component, multi-target, and multi-pathways [4-5].

Comfrey is a plant of the genus Comfrey in the family Comfreyaceae, derived from the dried roots of Xinjiang comfrey and Inner Mongolia comfrey in the family Comfreyaceae. In recent years, various effects of comfrey such as anticancer, anti-inflammatory, antiviral, antitumour, and antioxidant have been confirmed [6-10]. Among these, the anticancer research on comfrey is becoming more and more profound, and it has become one of the hotspots of anticancer research in traditional Chinese medicine. Modern pharmacological studies have found that comfrey can inhibit the growth and proliferation of cancer cells by inducing programmed apoptosis, attenuating cancer cell invasion, regulating the activity of cellular pathways, inducing ROS, and inhibiting angiogenesis, etc. [11], resulting in the effective treatment of hepatocellular carcinoma [12], colorectal carcinoma [13], cervical carcinoma [14] and thyroid carcinoma [15]. However, the target and molecular mechanism of comfrey in the treatment of lung cancer has not been elucidated. Therefore, this

experiment takes comfrey as the research object, explores the components, targets, and pathways of comfrey based on network pharmacology and molecular docking, obtains the key genes of therapeutic effects by screening, and verifies the binding degree of them with the active ingredients by molecular docking, to explore the mechanism of action of comfrey in the treatment of lung cancer and the accuracy of the predicted targets, and provide more research ideas and theoretical basis for its clinical application. This will provide more research ideas and a theoretical basis for its clinical application.

2 MATERIALS AND METHODS

2.1 Screening of Comfrey compounds and related targets

'Comfrey' was entered into the TCMSP database (https://www.tcmsp-e.com/), and screening values of oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were set for the search. The chemical structure of the active ingredient was found by searching the corresponding SMILE formula in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the MOL2 file was downloaded from TCMSP for those ingredients not included in the PubChem database, the MOL2 files were converted to SMILES files using 0pen Babel software, and the chemical structures of the major components were imported into Swiss Target Prediction (http://swisstargetprediction.ch/) to screen for the corresponding targets of the eligible major compounds, excluding targets without corresponding gene names and duplicate targets. The resulting compounds and drug targets were integrated to provide a drug compound and drug target dataset.

2.2 Collection of disease targets and acquisition of overlapping targets and drawing of Venn diagrams

The GeneCards (http://www.genecards.org/) and OMIM (www.omim.org) databases were searched using the keyword 'lung cancer' to obtain relevant targets for lung cancer. In addition, the GeneCards database targets were further screened for relevance with a relevance score ≥17.5315732955933 (the median was taken multiple times). The targets of the two databases were merged and the duplicate targets were deleted to obtain the lung cancer targets. The comfrey drug targets and lung cancer disease targets obtained were imported into the Microbiotics online mapping tool (https://www.bioinformatics.com.cn/) to obtain the intersection targets, and the Venn diagrams of the intersection of comfrey drug targets and disease targets were generated.

2.3 Protein-protein interaction network (PPI) construction and core target screening

The 147 overlapping targets were entered into the String database (https://string-db.org/cgi/input.pl), the species was restricted to 'Homo sapiens', the interaction threshold was selected as 'medium confidence (0.400)' and the colour shades of the edges were set to reflect the degree of relationship. The interaction threshold was selected as 'medium confidence (0.400)' and the colour shades of the edges were set to reflect the degree of relationship so that the PPI network relationship graph was obtained and the data results were exported in TSV format. The output data were imported into Cytoscape 3.9.1 to visualise the PPI network, and the topological parameters of the PPI network were analyzed using the CytoNCA plug-in to calculate the degree, mediacy, and proximity of each node to further define the key targets of comfrey in the treatment of lung cancer. The area size, colour depth, and order of nodes (target genes) in the network were regulated according to the degree value order, the higher the degree value, the larger the node area, the darker the colour, the deepest being red, and the combined score size as indicated by the thickness of the connecting lines to obtain the PPI relationship visualisation network to determine the core targets of comfrey in the treatment of lung cancer.

2.4 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways Enrichment analysis

The overlapping targets were imported into the DAVID database (https://david.ncifcrf.gov/), and the species was selected as 'Homo sapiens', GO analysis and KEGG pathway analysis was performed, and the data were imported into the Bioinformatics website (http://www. The data were imported into the Bioinformatics website (. bioinformatics.com.cn/), and the top 10 entries with the highest degree of enrichment were filtered according to the order of PV value to generate a bubble chart for GO enrichment analysis; the top 25 entries with the highest degree of enrichment were filtered according to the order of PV value to generate a bar chart for KEGG enrichment analysis.

2.5 Traditional Chinese Medicine Active Ingredient Disease Target Network Construction

To obtain the targets of comfrey for lung cancer treatment, use Cytoscape 3.9.1 software to construct the visual network of traditional Chinese medicine, ingredients, diseases, and overlapping targets, and use Network Analyzer to perform network analysis, the higher the network degree value, the more the active ingredient is associated with a greater number of targets. The importance of the compounds in the treatment of lung cancer was evaluated by the degree value according to the derived node table.

2.6 Molecular docking

Lithospermidin A, lithospermidin B, [(1R)-1-(5,8-dihydroxy-1,4-dioxo-2-naphthyl)-4-methyl-pent-3-enyl] propanoate, the core drugs obtained in 2.5, were used as key compounds and combined with the AKT1, EGFR, TNF, the core targets obtained in 2.3, respectively, were subjected to docking treatment to obtain the docking binding energy. The PDB file of the core target 3D structure was obtained from the RCSB-PDB database (https://www.rcsb.org/) for the receptor;

the core drug 3D structure was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for the ligand. Binding energy was counted after dehydrogenation and hydrogenation of receptor ligands, pdbqt format conversion, grid, docking, run, and analysis using AutoDockTools-1.5.7 software, and default docking parameters were used for docking. The docked conformation with the highest output score was taken as the binding conformation and Pymol was used for 3D visualisation of the molecular docking results.

3 RESULTS

3.1 Comfrey major constituents, active compounds, and their predicted targets

A total of 51 compositions of comfrey were screened in the TCMSP database, and 12 active compositions (lithospermidin A, acetylshikonin, ethyl oleate (NF), sitosterol, 1-methoxyacetylshikonin, etc.) were found with OB ≥ 30% and DL ≥ 0.18 at the same time, as shown in Table 1. The potential targets of comfrey were predicted using the SwissTargetPrediction database, and 904 comfrey-related targets were obtained.

Table 1: Comfrey Active Ingredient Information

Serial Number	Mol ID	Molecule Name	OB (%)	DL
ZC1	MOL001494	Mandenol	41.99	0.19
ZC2	MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	33.54	0.42
ZC3	MOL002883	Ethyl oleate (NF)	32.39	0.19
ZC4	MOL000359	Sitosterol	36.91	0.75
ZC5	MOL007714	1-methoxy acetyl shikonin	73.09	0.29
ZC6	MOL007715	[(1R)-1-(5,8-dihydroxy-1,4-dioxo-2-naphthyl)-4-methyl-pent-3-enyl] propanoate	54.64	0.29
ZC7	MOL007716	Acetylshikonin	62.38	0.26
ZC8	MOL007722	Isoarnebin 4	64.79	0.19
ZC9	MOL007728	Lithospermidin A	75.07	0.38
ZC10	MOL007734	5-[(E)-5-(3-furyl)-2-methyl-pent-2-enyl]-2,3-dimethoxy-p-benzoquinone	61.79	0.23
ZC11	MOL007735	Des-O-methyllasiodiplodin	30.11	0.20
ZC12	MOL007736	Lithospermidin B	60.47	0.38

3.2 Lung cancer-related target screening and overlapping target Venn diagrams

The GeneCards database and the OMIM database were searched for lung cancer-related targets, and 1670 targets were obtained by screening with a relevance score ≥17.5315732955933 in the GeneCards database and 66 targets were obtained from the OMIM database, and 1717 lung cancer-related targets were obtained by aggregating and de-weighting the information. After aggregation and de-weighting, a total of 1,717 lung cancer-related targets were obtained; overlapping with 904 drug targets of comfrey, 147 key targets were obtained, including EGFR, TERT, ALK, BRAF, MET, etc., i.e. the potential targets of comfrey in the treatment of lung cancer. To better illustrate the logical connection between drug targets and disease targets, this experiment uses the Microbiology Letter online mapping tool to create a Venn diagram of the intersection of drug targets and disease targets, which intuitively shows the characteristic relationship between each set, see Figure 1.

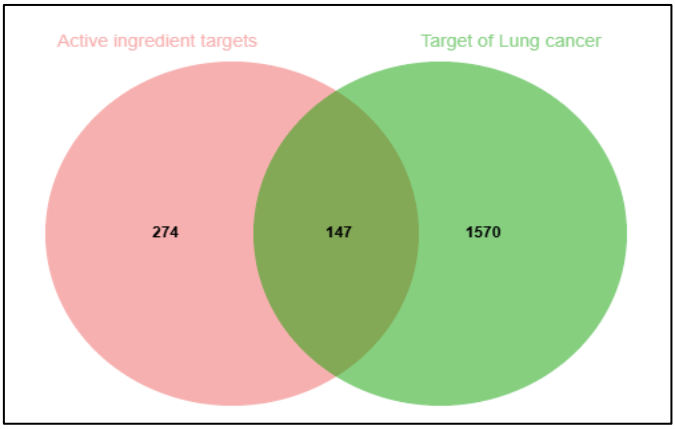

Figure 1: Intersecting targets Venn diagram

3.3 Results of the PPI network analysis

The 147 target genes mentioned above were uploaded into the String database, and the PPI data were obtained according to the set conditions, and then imported into Cytoscape software to draw the PPI network, see Figure 2. There are 147 nodes and 2,646 edges in the PPI network as shown in the figure. The target closest to the central region of the network is connected to multiple targets. The size and colour of the nodes were set according to the Degree value, the larger

the Degree value, the larger and darker the node; the strength of the relationship between the nodes was indicated by the thickness of the edges, and the thicker the line of the edges, the higher the value of the combined score. After sorting, 10 target proteins such as AKT1, EGFR, TNF, CASP3, HSP90AA1, etc. are the top ten sorted by Degree value (see Table 2), and it can be considered that these target proteins play an important role in the treatment of lung cancer by Zizyphus vulgaris.

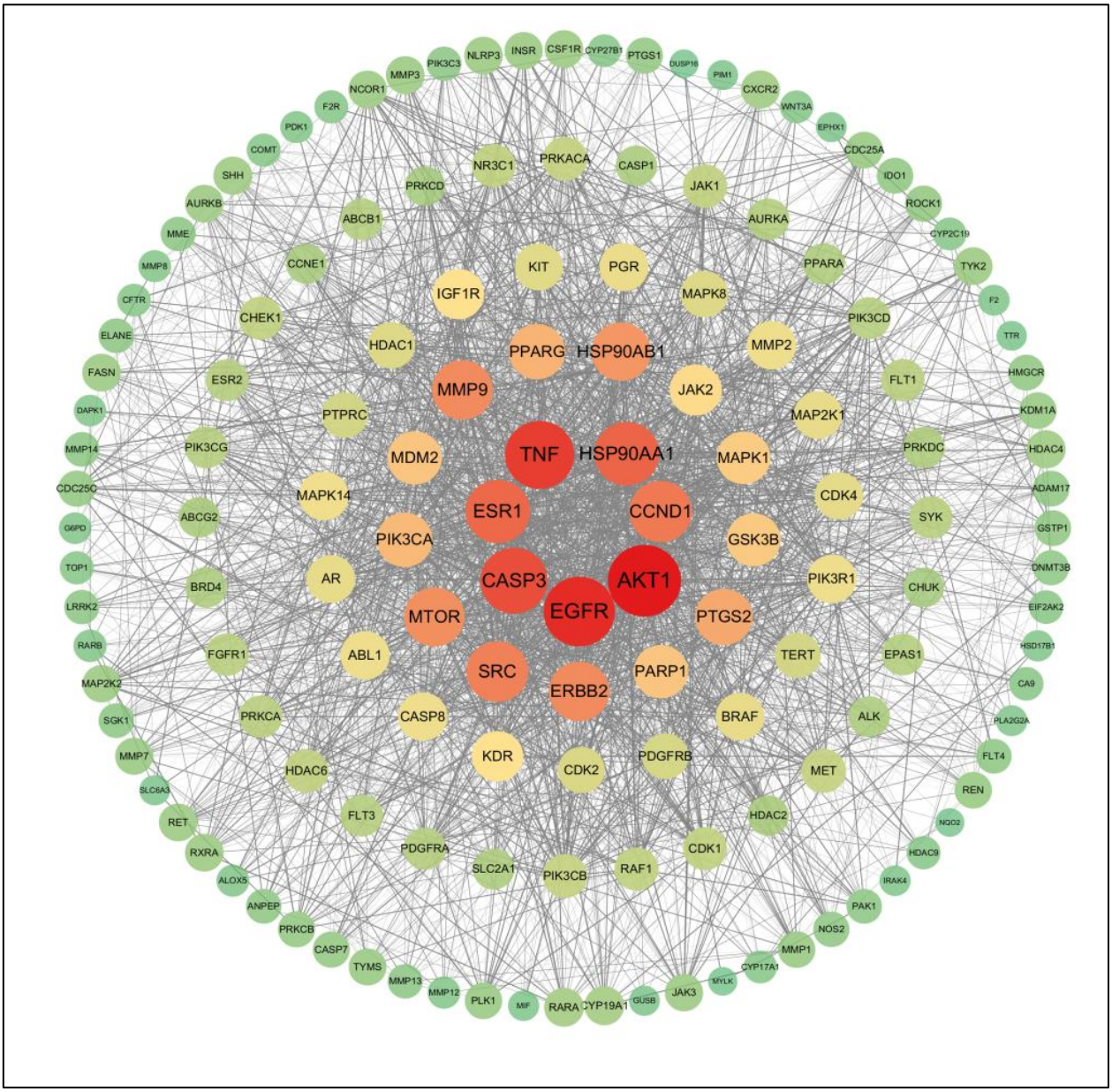

Figure 2: PPI for comfrey in lung cancer Webmap

Table 2: Top 10 target proteins of comfrey in lung cancer treatment

Target	Degree	Betweenness	Closeness
AKT1	116	1690.12	0.83
EGFR	111	1280.54	0.81
TNF	106	1384.42	0.78
CASP3	101	740.50	0.76
HSP90AA1	95	691.89	0.74
ESR1	94	718.29	0.74
CCND1	89	501.07	0.72
SRC	87	442.63	0.71
MMP9	84	502.90	0.70
ERBB2	84	376.08	0.70

3.4 Results of Pathway Enrichment Analysis

In the DAVID database, the potential targets of comfrey for lung cancer treatment were enriched by GO and KEGG pathways, and the GO function was enriched in 690 biological processes (BP), 96 cellular components (CC), and 139 molecular functions (MF). The biological process (BP) includes phosphorylation, protein phosphorylation, protein autophosphorylation, peptidyl-tyrosine phosphorylation, multicellular organism development, etc.; the cellular component (CC) includes cytosol, cytoplasm, receptor, and cellular function. Cellular Composition (CC) includes cytosol, cytoplasm, receptor complex, nucleoplasm, plasma membrane, etc; Molecular function (MF) includes ATP binding, protein kinase activity, protein serine kinase activity, protein tyrosine kinase activity, protein serine/threonine kinase activity, etc. The bubble diagram of the GO enrichment analysis is shown in Figure 3, where the vertical axis is the descriptive information of the relevant enriched entries and the horizontal axis is the false rejection rate of the relevant entries. The size of the bubble represents the number of enriched genes, the more enriched gene entries, the larger the bubble; the colour corresponds to the P-value, from large to small, corresponding to blue to red, the smaller the P-value, the higher the enrichment degree and the more important the entries are.

A total of 166 pathways were enriched by KEGG, and the top-ranked pathways according to the P value mainly included pathways in cancer, prostate cancer, PI3K-Akt signalling pathway, endocrine resistance, proteoglycans in cancer, etc. The bar graph of the KEGG enrichment analysis is shown in Figure 4, the vertical axis is the description information of the enriched pathways, and the horizontal axis is the number of differentially expressed genes of the respective pathways. The colours correspond to the P-values, from large to small, corresponding to blue to red, the smaller the P-value, the higher the enrichment and the more important the pathway.

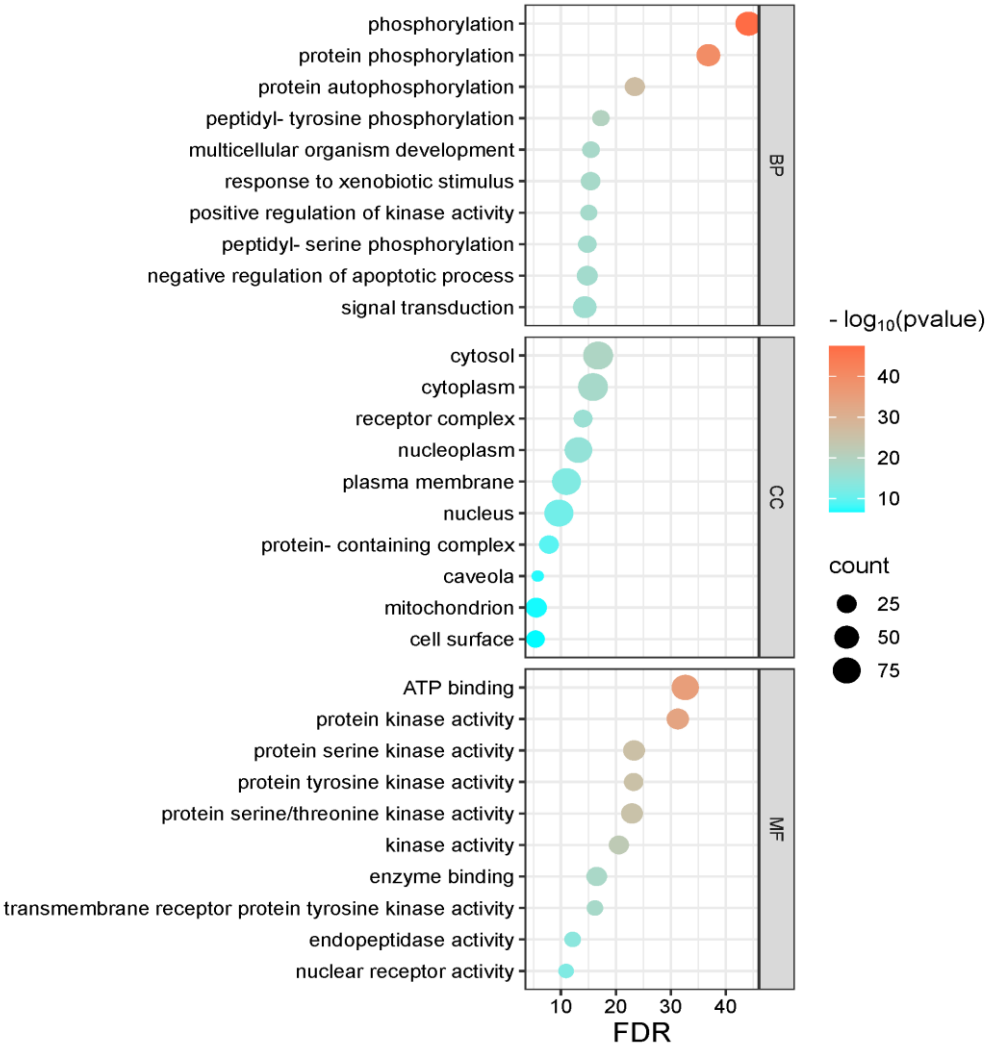

Figure 3: GO enrichment analysis bubble diagram

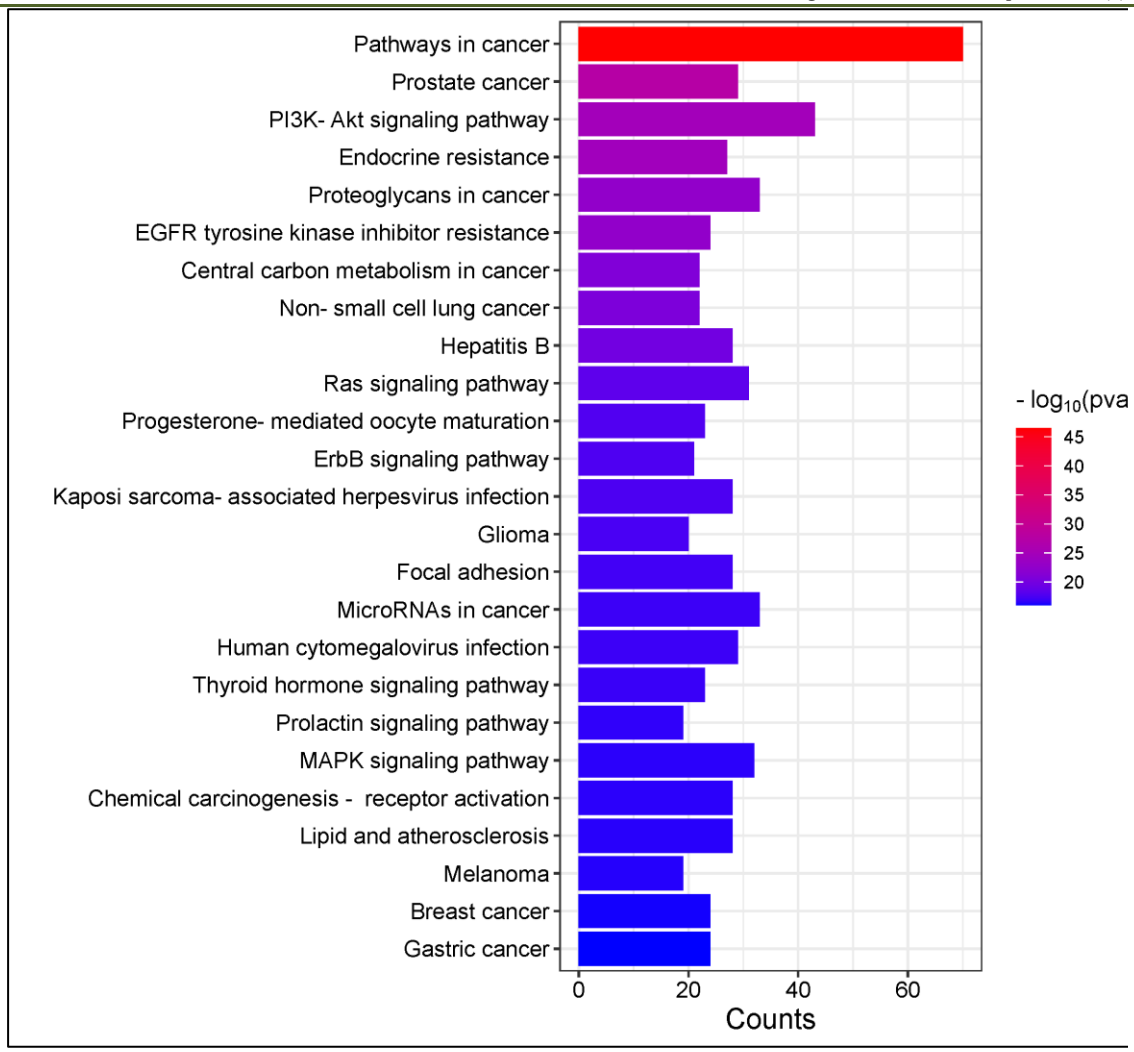

Figure 4: Bar graph of KEGG enrichment analysis

3.5 Analysis of the construction of the ' Traditional Chinese Medicine Active Ingredient Disease Target Network'

Cytoscape software was used to construct the 'TCM-active ingredients-disease-targets' network diagram (see Figure 5). The analysis showed that 12 compounds corresponded to 147 overlapping targets, with 161 nodes and 545 edges. The Network Analyzer analysis showed that the average degree value was 31.1, and the following 7 active ingredients with higher than average degree values were the key ingredients of Ziziphus for the treatment of lung cancer, as shown in Table 3.

Table 3: Above average levels of comfrey compounds

Serial Number	Molecule Name	Degree	Betweenness Centrality	Closeness Centrality
ZC9	Lithospermidin A	58	0.04681	0.44693
ZC12	Lithospermidin B	54	0.04215	0.44199
ZC6	[(1R)-1-(5,8-dihydroxy-1,4-dioxo-2-naphthyl)-4-methyl-pent-3-enyl] propanoate	47	0.03316	0.42553
ZC7	Acetylshikonin	44	0.03004	0.42105
ZC5	1-methoxy acetyl shikonin	42	0.02949	0.41667
ZC11	Des-O-methyllasiodiplodin	41	0.03281	0.41451
ZC10	5-[(E)-5-(3-furyl)-2-methyl-pent-2-enyl]-2,3-dimethoxy-p-benzoquinone	40	0.03099	0.41237

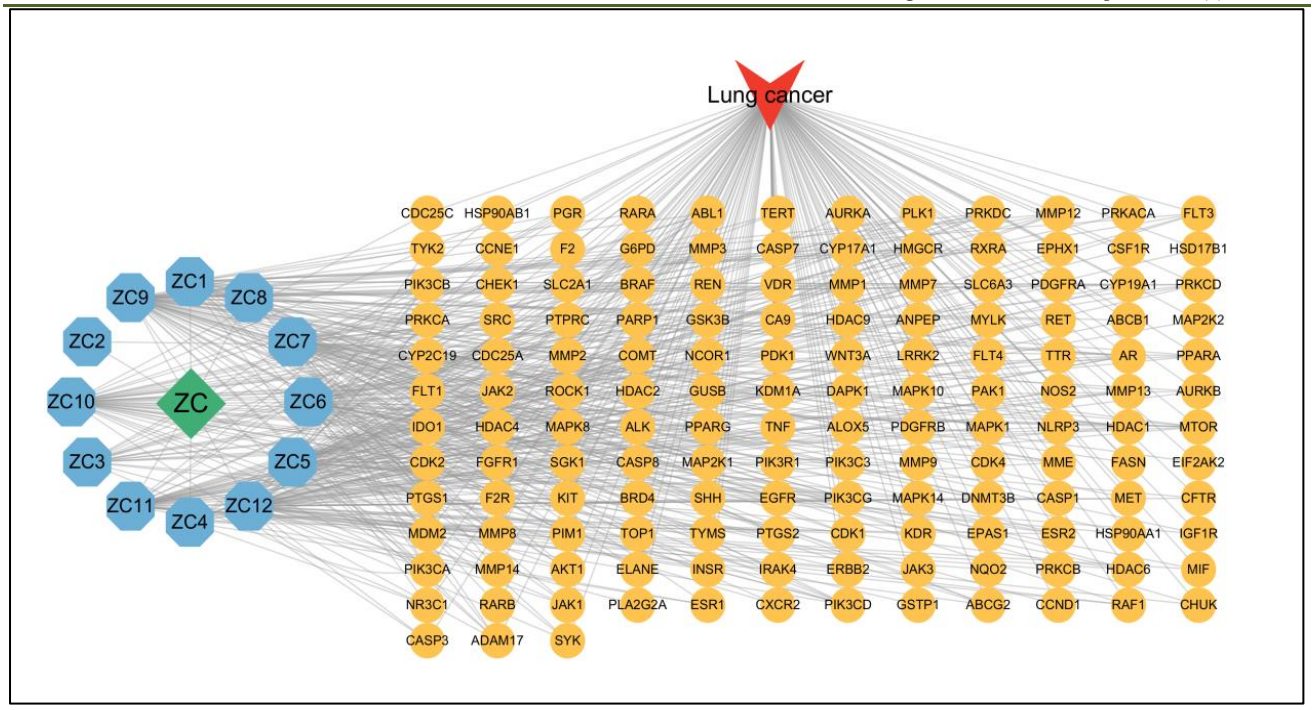

Figure 5: Comfrey-Active Ingredient-Disease-Target visualisation network map

Note: ZC stands for Ziziphus, green diamond for the herb Ziziphus, blue octagon for the 12 active ingredients of Ziziphus, orange circle for the 147 common targets, and red V for the disease.

3.6 Validation of Molecular Docking

To validate the accuracy of the network pharmacological prediction results, the core targets obtained in 2.3 were molecularly docked to the core compounds obtained in 2.5. It is generally accepted that the lower the docking score, the stronger the docking activity. The thermogram of molecular docking binding energy is shown in Figure 6, the darker the colour, the better the docking effect. The docking results of the core target and its lowest binding energy drug were visualised using Pymol software (Figures 7, 8, and 9), and hydrogen bonds were formed between the drugs and the targets, suggesting that they have good binding activity. 53; EGFR formed hydrogen bonds with 3 amino acid residues GLN-982, VAL-980 and MET-908 in Lithospermidin B; and TNF formed hydrogen bonds with 5 amino acid residues ASN-34, ALA-33, SER-147, GLN-149 and VAL-150 in Lithospermidin A. The AKT1 was also shown to have good binding activity. The results showed that comfrey has a good therapeutic or ameliorative effect on lung cancer, especially the lithospermidin A and lithospermidin B components.

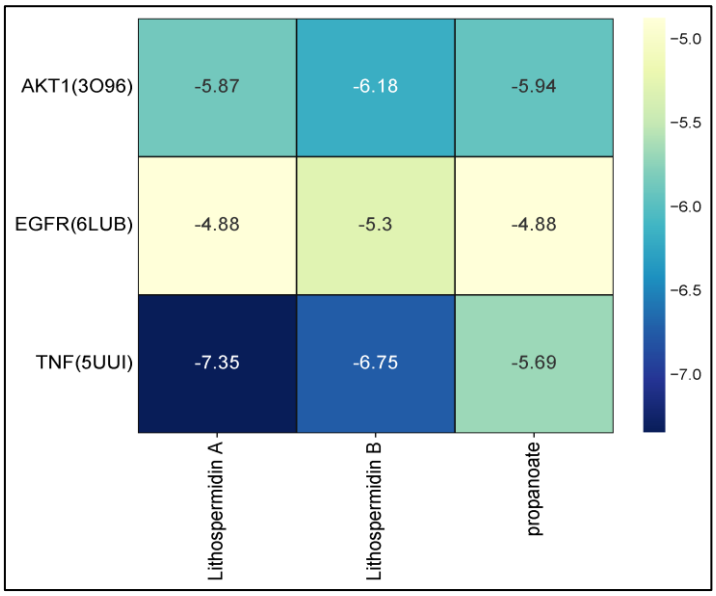

Figure 6: Heatmap of molecular docking scoring of compounds with key targets in Comfrey

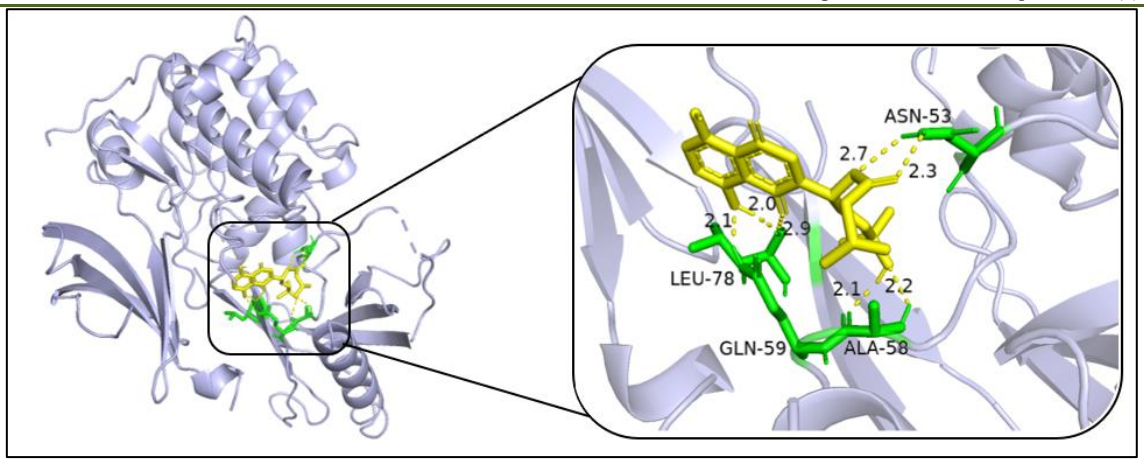

Figure 7: AKT1and Lithospermidin B

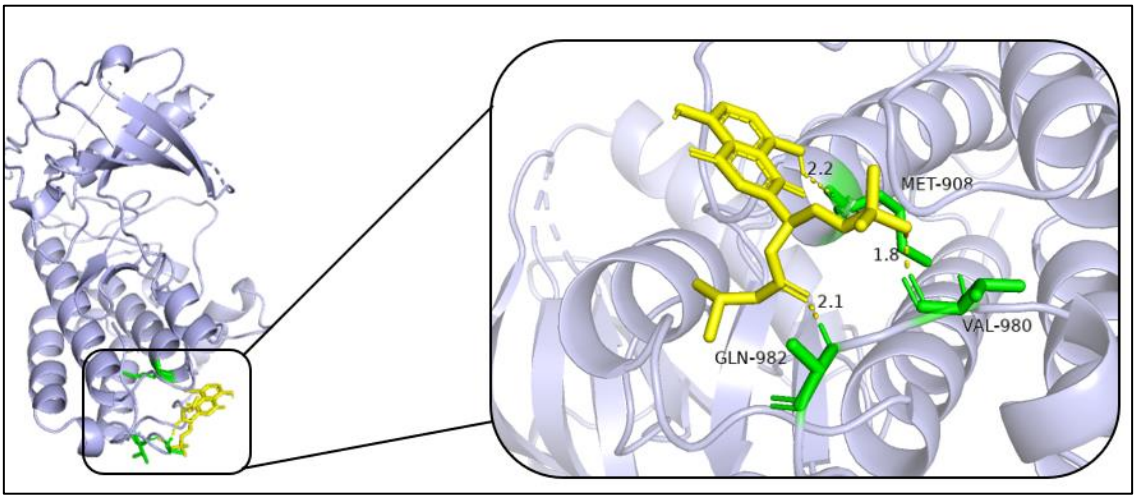

Figure 8: EGFR and Lithospermidin B

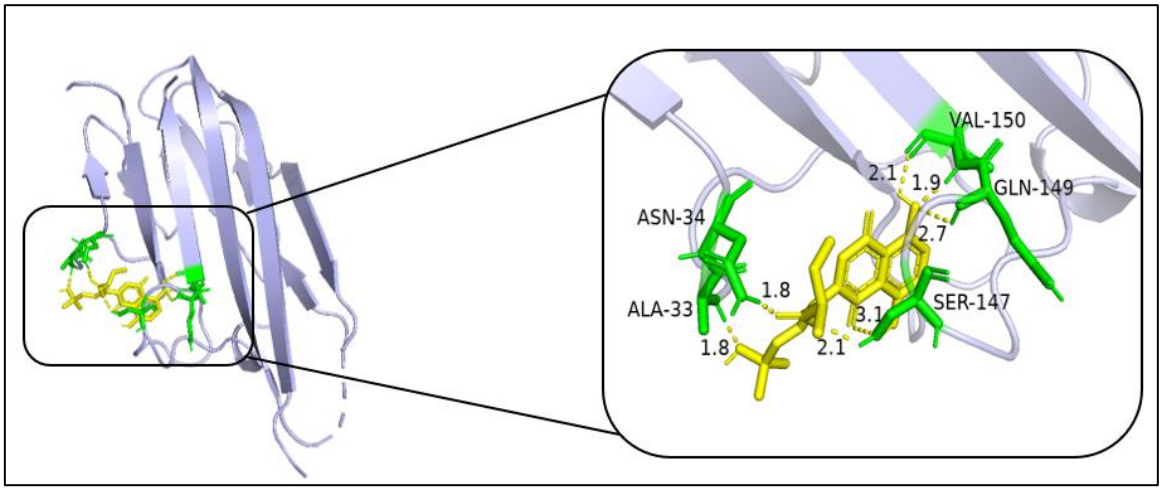

Figure 9: TNFand Lithospermidin A

4. DISCUSSION

Lung cancer has a high incidence, morbidity, and mortality rate, mainly manifested by chest pain, cough significant reduction in physical fitness, etc. Factors such as air pollution, cigarette smoking, ionizing radiation, and heredity may increase the risk of developing lung cancer. Treatment is mainly based on targeted therapy, immunotherapy, chemotherapy, etc. [16], but is limited by the general condition of patients with advanced lung cancer, response to induction therapy, gene mutation status, etc., and the clinical effect of some patients is not obvious. Traditional Chinese medicine shows efficacy due to its multi-component, multi-target, and multi-dimensional mechanism of

action, and the use of traditional Chinese medicine comfrey in the treatment of lung cancer not only reduces toxic side effects but also improves the quality of life of patients.

In this study, the mechanism of anti-lung cancer action of comfrey was analyzed from the perspective of relevant targets, overlapping genes, and pathways using network pharmacology and molecular docking methods, databases, and related software. The results showed that lithospermidin A, lithospermidin B, [(1R)-1-(5,8-dihydroxy-1,4-dioxo-2-naphthyl)-4-methyl-pent-3-enyl] propanoate, the major active constituents of comfrey. Acetylshikonin, 1-methoxyacetylshikonin, etc. are all strongly associated with the disease. In particular, lithospermidin A, lithospermidin B, acetylshikonin, and 1-methoxyacetylshikonin are naphthoquinones with good anti-tumor, anti-inflammatory and other effects, which have high medicinal value [17-18]. PPI network analysis showed that the node degree values of AKT1, EGFR, TNF, CASP3, and other genes have node degree values >100, which are significantly higher than other targets, indicating that the core potential targets of comfrey against lung cancer may be the above targets. AKT1 is the substrate of PI3K, which mainly regulates cell proliferation and apoptosis, and reduces the level of mRNA and protein expression. EGFR may be a new option for treating lung cancer. Since FOLKMAN first proposed the theory of anti-angiogenesis, anti-angiogenic therapy has been progressively developed, with some studies finding that EGFR (epidermal growth factor receptor) mutant NSCLC cells can promote VEGF (vascular endothelial growth factor) expression [19]. TNF-α is an important cytokine synthesised and secreted by macrophages and monocytes in vivo, and TNF-αmay has an inhibitory effect on tumour growth and promotes tumour necrosis, and some studies have shown that TNF-α can regulate lung cancer cell proliferation, apoptosis, and invasion by modulating the NF-κB/PXR inflammatory pathway [20]. These further support the results of this experiment. The key targets of Comfrey all play very important roles in the treatment of lung cancer.

GO enrichment revealed that comfrey treatment of lung cancer mainly involves phosphorylation, cytosol, ATP binding, etc. This may be related to the more complex pathogenesis of lung cancer, suggesting that comfrey may intervene in the process of lung cancer at different levels and through multiple pathways. The results of KEGG enrichment analysis showed that the possible pathways of comfrey for the treatment of lung cancer included pathways in cancer, prostate cancer, PI3K-Akt signalling pathway, endocrine resistance, proteoglycans in cancer, and the results predicted that the obtained therapeutic targets of comfrey were more enriched in cancer pathways, suggesting that comfrey may prevent cancer by inhibiting cancer pathways. In conclusion, the present study analyzed and predicted the active compounds and important targets of comfrey in the treatment of lung cancer using network pharmacology, and preliminarily verified its targets using molecular docking technology. The preliminary results of this study suggest that comfrey may treat lung cancer through 'multi-targets and multi-pathways', which may provide a reference for further research into the mechanism of action.

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