

Mechanisms of Tibetan Medicine Sophora Moorcroftiana Alkaloids for Treatment of Lung Cancer based on the Network Pharmacology, Molecular Docking and cell Experimental Verification

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Abstract

Background

Leguminous *Sophora moorcroftiana* (SM) is a genuine medicinal material in Tibet. Many research results have reveal the *Sophora moorcroftiana* alkaloids (SMA), as the main active substance, have a wide range of effects, such as antibacterial, antitumor and antiparasitic effects. However, there are few reports on the inhibition of lung cancer (LC) and its inhibitory mechanism, and the pharmacological mechanism of SMA is still unclear, Therefore, exploring its mechanism of action is of great significance.

Methods

The SMA active components were obtained from the literature database. Whereas the corresponding targets were screened from the PubChem and PharmMapper database, UniProt database were conducted the correction and transformation of UniProt ID on the obtained targets. The GeneCards and OMIM databases identified targets associated with LC. Venny tools obtained the intersection targets of SMA and LC. R language and Cytoscape software constructed the visual of SMA - intersection targets – LC disease network. The intersection targets protein-protein interaction (PPI) network were built by the STRING database. The functions and pathways of the common targets of SMA and LC were enriched by gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Subsequently, molecular docking And A549 cells vitro experiment were performed to further validate our finding.

Results

We obtained six kinds of alkaloids in SM, 635 potential targets for these compounds, and 1,303 genes related to LC. SMA and LC intersection targets was 33, including ALB, CCND1, ESR1, NOTCH1 and AR. GO enrichment indicated that biological process of SMA was mainly involved in the positive regulation of transcription and nitric oxide biosynthetic process, and DNA-templated, etc. Biological functions were mainly involved in transcription factor binding and enzyme binding, etc. Cell components were mainly involved in protein complexes, extracellular exosome, cytoplasm and nuclear chromatin, etc, Which may be associated with its anti-LC effects. KEGG enrichment analysis showed that main pathways involved in the anti-LC effects of SMA, including pathway in cancer, non small-cell lung cancer, p53, PI3K-Akt and FOXO signaling pathways. Molecular docking analyses revealed that the six active compounds had a good binding activity with the main therapeutic targets 2W96, 2CCH and 1O96. Experiments in vitro proved that SMA inhibited the proliferation of LC A549 cells.

Conclusions

results of the present study, we have successfully revealed the SMA compounds had a multi-target and multi-channel regulatory mechanism in treatment LC, These findings provided a solid theoretical reference of SMA in the clinical treatment of LC.

Introduction

Lung cancer (LC), as an exceedingly significant public health problem in developing and developed countries, has a high incidence and mortality rate, and it is one of the most common malignant tumors in clinical practice.^[1] In recent years, the incidence and mortality rate of LC patients have been showing an upward trend. Currently, although the surgery treatment for LC can be effective, the overall prognosis is poor.^[2] With its advantages of low toxicity, and multi-component and multi-target mechanism, traditional Chinese medicine can not only provide new and effective candidate drugs for the treatment of cancer diseases, but also has a good application prospect and research value. The combination of traditional Chinese and Western medicine can effectively treat LC and significantly improve the efficiency of treatment.^[3] Therefore, screening the effective components of traditional Chinese medicine as effective drugs for treating LC is of great significance.

Leguminous *Sophora moorcroftiana* (SM) is a genuine medicinal material in Tibet. Its alkaloids have various biological activities, including bacteriostasis, antitumor, and anti-parasite.^[4] A continuous in-depth study on the biological activity of SMA found that the inhibitory effect of alkaloid extracts on the proliferation of lung adenocarcinoma cells SPC-A-1 and GLC-82 showed significant dose and time effects. Still, the mechanism of action was unclear.^[5]

As a new analytical technology, network pharmacology analysis can integrate biological multicomponent and multitarget activities, because it is consistent with the integrity and systematicness of traditional Chinese medicine.^[6, 7] This discipline is suitable for the in-depth mining of potential active ingredients in traditional Chinese medicine, especially for screening and exploring the mechanism of the effective components of a single drug.^[8] Molecular docking technology can predict the binding mode and binding energy of the receptor and ligand by knowing the structural characteristics of the receptor and ligand and the interaction force between them, and further screen out the optimal binding mode of drug molecular ligands and docking receptors. This technology can not only reveal the molecular mechanism of receptor-ligand binding, but also contribute to the pharmacology of traditional Chinese medicine compounds and single drugs. Therefore, This study aim was to apply network pharmacology, molecular docking and cell Experimental Verification to reveal the underlying mechanism by which SMA effects for LC, and to predict the main active compounds and core targets of SMA for the treatment of LC, As well as provide a scientific basis for the biological activity interpretation of LC.

Materials and Methods

Potential active ingredients target and gene name tagging of SMA components

'*Sophora Moorcroftiana*' was used as a search term in CNKI literature database (<https://www.cnki.net/>) and Chemical constituents confirmed by NMR and MS were screened out from the literature. All the chemical components and structural formulas were obtained from the ChemDraw 17.1.0 software and the use of the Search option 'Find structure from name at ChemACX. Com'. PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) was used to search (3D) chemical structure of SMA, the 3D structure was saved as an SDF file and imported into PharmMapper database (<http://www.lilab-ecust.cn/pharmmapper>) for prediction, with the use of the keywords 'druggable pharmaceutical models' to acquire the potential targets of chemical components, the UniProt ID was corrected and transformed by using the UniProt database (<https://www.uniprot.org/uniprot>).

Prediction of target genes for LC

The search term 'Lung cancer' was used to search the GeneCards database (<https://www.genecards.org/>) and OMIM database (<https://omim.org/>), GeneCards database results were screened with correlation score > 20. OMIM database results were screened with 'gene Map' option, Finally, the two database valuable targets were merged, and the repeated targets were deleted to screen LC-related potential valuable targets.

Construction of PPI network and Screening the therapeutic intersection target for LC

In this study, venny 2.1.0 online tool (<https://bioinfogp.cnb.csic.es/tools/venny/>) combined with R 3.6.3 software was used to construct the visualization of drug components, disease targets and intersection targets. The interaction regulatory network of the drug components and disease target was constructed by using Cytoscape 3.7.0 software. The interaction regulatory network of the intersection targets was constructed by using the STRING database (<https://string-db.org/>). The intersection targets were selected from the PPI network.

GO and KEGG pathway enrichment analysis

To further explore the mechanisms of SMA for LC treatment, the intersection targets was input into the DAVID database (<https://david.ncifcrf.gov/>), and the selected identifier was set as 'official gene symbol'. The Biological Process (BP), Cell Component (CC), Molecular Function (MF) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were obtained and analyzed. Finally, the GO functions and KEGG pathway were visualized by using R 3.6.3 software.

Molecular docking

The intersection targets proteins of LC, which is the active component of SMA, was ligated. The specific operation includes the following aspects: (1) The structure of SMA active components was constructed

by using ChemOffice 2017 software based on ChemDraw Professional, and the energy of the obtained structure was minimized in Chem 3D; (2) The 3D structures of the core target protein was obtained from the PDB database (<http://www.rcsb.org/>); (3) To separate the original ligand and remove the hydrone and other inactive ligands from the core target protein by using PyMol software; (4) Autodock was used to process and dock the protein receptors and molecular ligands. Pymol was used to visualize the docking results.

Cell experiment verification

Obtaining SMA extract

In the early experiment, the extraction scheme of SMA was optimized. The extraction was carried out under 75% ethanol concentration, ultrasonic temperature 55°C solid-liquid ratio 1 g/mL: 30 g/mL, ultrasonic power 150 W and ultrasonic time 50 min. After the extract is filtered and concentrated in a rotary evaporator, it is dried in a vacuum drying oven to obtain the dry powder of the section of SM.

Cell culture and proliferation assay

LC A549 cells were obtained from the Lanzhou General Hospital of Lanzhou Military Region. LC A549 cells in logarithmic growth stage were taken and their concentration was adjusted to 2×10^5 pieces/mL, 100 μ L per well, inoculated on 96 well plates, cultured at 80% confluence, 5% carbon dioxide and 37°C cultured 24 h. Then add the solution of SMA at the concentrations of 3000, 1500, 750 and 375 μ g/mL, six multiple wells at each concentration, and then treated for 12, 24 and 36 h. After cells were incubated with CCK-8, and the optical density (OD) was measured at the absorption wavelength of 450 nm. According to the formula: cell proliferation inhibition rate = (control hole - experimental hole) / (control hole - blank hole) $\times$ The inhibition rate of cells was calculated at 100%.

Statistical analysis

All data were expressed as the mean $\pm$ standard deviation, and the measurement results were analyzed with SPSS 26.0 statistical software performed one-way ANOVA. Statistical significance was defined as P-values, $P \leq 0.05$ was considered statistically significant, $P \leq 0.01$ was considered statistically have extremely significant.

Results

Drug ingredients of SMA and obtaining the SMA component- LC disease potential targets

According to the collected literature, matrine, oxymatrine, sophocarpine, oxysophocarpine, sophoridine and cytosine component were obtained.^[9, 10] The selected six component was confirmed by NMR and MS. The SMA component targets obtained from the PharmMapper database were corrected and transformed by using the UniProt database. Repeats and false positives were removed. A total of 635

potential targets were obtained, and 1303 LC targets were obtained from the GeneCards and OMIM database. Thirty-three intersection targets existed between SMA and LC, with oxysophocarpine and oxymatrine having the most target numbers in LC (Fig. 1).

Construction of SMA component and LC disease intersection targets network

The control network of the drug component target disease was constructed by using Cytoscape 3.7.0. The node represents the SM and SMA component, SMA component and LC disease target, while the edge shows the correlation between the drug component and action targets (Fig. 2).

Construction of PPI interaction regulatory network of SMA and LC intersection targets

The PPI interaction regulatory network of the intersection target constructed by the STRING database contains 33 nodes and 96 edges. The average node degree value is 5.82, and the local clustering coefficient is 0.53. The nodes in the network represent the targets, the edges between nodes represent protein and protein interactions, and the different colors represent different interactions (Fig. 3A). the targets with higher degree values were ALB (degree: 17), CCND1 (degree: 14), ESR1 (degree: 13), NOTCH1 (degree: 13), AR (degree: 12), CDK2 (degree: 11), HSP90AA1 (degree: 11) (Fig. 3B) .

Go and KEGG enrichment analysis

A total of 129 GO entries were enriched in the DAVID databases, 76 of which were BP, 31 were MF, and 22 were CC, accounting for 59%, 24% and 17%, respectively. The most significant BP involves the positive regulation of transcription, DNA-templated (GO: 0045893), positive regulation of nitric oxide biosynthetic process (GO: 0045429) and prostate gland development (GO: 0030850)(Fig. 4A). The most significant MF involves transcription factor binding (GO: 0008134), enzyme binding (GO: 0019899) and identity protein binding (GO: 0042802)(Fig. 4B). The most significant CC involved protein complex (GO: 0043234), extracellular exosomes (GO: 0070062), cytoplasm (GO: 0005737) and nuclear chromatin (GO: 0000790) (Fig. 4C). In addition, 21 KEGG pathways were enriched. The most significant pathways were pathways in cancer (hsa05200) and the PI3K-Akt signaling pathway (hsa04151), followed by the p53 signaling pathway (hsa04115), glutathione metabolism (hsa00480), non-small cell lung cancer (hsa05223) and prostate cancer (hsa05215) (Fig. 4D). And the SMA- LC intersection targets KEGG enrichment pathway and gene name(Table 1).

Table 1
SMA- LC
intersection
targets
KEGG
enrichment
pathway
and gene
name

NO	Term	count	P-Value	Genes
1	hsa05200: 21Pathways in cancer	10	9.90×10^{-4}	E2F1 AR CCND1 HSP90AA1 BCR RAC1 RARA STK4 TRAF4 CDK2
2	hsa05215: Prostate cancer	5	3.50×10^{-4}	E2F1 AR CCND1 HSP90AA1 CDK2
3	hsa00480: Glutathione metabolism	4	8.60×10^{-4}	ODC1 RRM1 IDH2 IDH1
4	hsa05223: Non-small cell lung cancer	4	1.30×10^{-3}	E2F1 CCND1 ALK STK4
5	hsa04115: p53 signalling pathway	4	2.50×10^{-3}	CCND1 THBS1 CDK2 TP73
6	hsa05222: Small cell lung cancer	4	4.90×10^{-3}	E2F1 CCND1 TRAF4 CDK2
7	hsa04151: PI3K-Akt signaling pathway	6	9.10×10^{-3}	CCND1 HSP90AA1 RAC1 THBS1 INSR CDK2
8	hsa05219: Bladder cancer	3	1.10×10^{-2}	E2F1 CCND1 THBS1
9	hsa04110: Cell cycle	4	1.20×10^{-2}	E2F1 CCND1 CCNA2 CDK2
10	hsa04068: FoxO signaling pathway	4	1.30×10^{-2}	CCND1 STK4 INSR CDK2
11	hsa05161: Hepatitis B	4	2.00×10^{-2}	E2F1 CCND1 CCNA2 CDK2
12	hsa05212: Pancreatic cancer	3	2.80×10^{-2}	E2F1 CCND1 RAC1
13	hsa05220: Chronic myeloid leukemia	3	3.40×10^{-2}	E2F1 CCND1 BCR
14	hsa05205: Proteoglycans in cancer	4	4.20×10^{-2}	CCND1 RAC1 ESR1 THBS1
15	hsa05203: Viral carcinogenesis	4	4.20×10^{-2}	CCND1 RAC1 CCNA2 CDK2

NO	Term	count	P-Value	Genes
16	hsa04914: Progesterone-mediated oocyte maturation	3	4.30×10^{-2}	HSP90AA1 CCNA2 CDK2
17	hsa01210: 2-Oxocarboxylic acid metabolism	2	6.00×10^{-2}	IDH2 IDH1
18	hsa04919: Thyroid hormone signaling pathway	3	7.30×10^{-2}	NOTCH1 CCND1 ESR1
19	hsa04152: AMPK signaling pathway	3	7.90×10^{-2}	CCND1 CCNA2 INSR
20	hsa05162: Measles	3	9.60×10^{-2}	CCND1 CDK2 TP73
21	hsa05206: MicroRNAs in cancer	4	8.00×10^{-2}	E2F1 NOTCH1 CCND1 THBS1

Molecular docking

Six alkaloids from SM were linked with CCND1 (2W96), CDK2 (2CCH) and E2F1 (1O96) targets. The docking of each active component and protein produces ten docking results. According to the principle of a lower binding energy corresponding to an increased possibility of interaction between protein and molecule and to a stable binding conformation, the conformation with the lowest binding energy of ligand and receptor is selected as the docking conformation. the results showed the binding energies of alkaloid components and target proteins were all less than -4 KJ/mol, indicating that the energetic drug molecules could bind to the target protein well. the binding sites of monomer components with protein amino acids and the binding length of hydrogen bonds (Fig. 5 Table 2).

Cell experiment in vitro

The results showed the alcohol extract of SMA acted on the A549 cell line, the cell proliferation of the cell line was inhibited to varying degrees. The adherent cells were rounded and floated under an inverted microscope, compared with the control group, the OD₄₅₀ of the drug treatment group changed most significantly at 24 and 36 h. As the drug concentration increased from 375 to 3000 µg/mL, The OD₄₅₀ absorbance gradually increased, and the OD₄₅₀ absorbance at 36 h was the highest. the cell inhibition rates of 375, 750, 1500 and 3000 µg/mL after 36 h treatment were 1.29%, 10.84%, 39.55% and 83.69%, respectively, which further shows that the SMA on LC A549 cells are time and dose-dependent (Fig. 6 Table 3).

Table 3
The inhibitory rate of SMA on LC A549 cells at different time and concentrations

Concentration (μg/mL)	Inhibition rate (%)		
	12 h	24 h	36 h
0	0	0	0
375	0.31	0.95	1.29
750	0.8	2.04	10.84
1500	11.21	23.06	39.55
3000	30.39	78.35	83.69

Discussion

In this study, we use network pharmacological approach to reveal the potential molecular mechanisms of SMA in LC. through Cytoscape software and PPI network 33 core targets were screened out from the SMA component and LC disease regulatory network. Among the intersection targets of SMA components and LC, oxysophocarpine (degree: 20) has the highest number, followed by oxymatrine (degree: 19). Previous research shows that oxymatrine, matrine, oxysophocarpine, sophocarpine, sophoridine and cytisine have different inhibitory effects on LC cell lines.^[11–15] such as oxymatrine can induce apoptosis of the human LC non-small cell LC cell line A549^[16, 17], and oxysophocarpine has an excellent inhibitory effect on liver cancer, colorectal cancer and other cell lines.^[18, 19]

By constructing the SMA and LC disease intersection targets gene interaction regulatory network, the targets with degree > 10 were selected as ALB, CCND1, ESR1, Notch1, AR, CDK2, HSP90AA1 and E2F1. CCND1 and CDK2 were both expressed in non-small cell lung cancer, and CCND1 was highly expressed in non-small cell lung cancer tissues (42%). The expression of CDK2 in non-small cell lines and tissue samples was negatively correlated with the expression of microRNA-597.^[20] The rs3213172C/T polymorphism of the E2F1 gene can be used as an effective biomarker for the genetic susceptibility of LC in the Chinese population.^[21] Therefore, the selected core targets can not only be used as markers of LC but may also become one of the key targets for the treatment of LC.

Based on the GO prediction of the BP pathway, MF pathway, CC pathway and KEGG signaling pathways by DAVID, the biological pathway is involved in the positive regulation of DNA template transcription; the molecular pathway is involved in transcription factor binding, enzyme binding and the same protein binding; and the cellular pathway is involved in protein complexes and exosomes. These results indicated that SMA might affect protein recombination by affecting protein complexes. The protein and exosomes were used to regulate the binding process of enzymes, thus affecting the transcriptional regulation of DNA templates. Among the 21 pathways, the essential pathways involved are the cancer pathway, PI3K-

Akt signaling pathway, p53 signaling pathway, glutathione metabolism, non-small cell lung cancer, prostate cancer and other pathways. As one of the signaling pathways of many cancer pathways, p53 and PI3K-Akt can negatively regulate the expression of the activator of transcription 3 (STAT3) in the lung cancer pathway, thus causing the overexpression of p53^[22], which then affects LC A549 cells. The PI3K-Akt signaling pathway plays an important role in the occurrence and metastasis of LC, and it is highly expressed in non-small cell LC.^[23] Therefore, the targets CDK2, CCND1, and E2F1 may be speculated to be the core target genes for the treatment of LC, and the p53 and PI3K-Akt signaling pathways have the most critical role in the treatment of LC through the SMA signaling pathway.

The molecular docking results showed that six active components of SMA combined well with the targets proteins 2w96, 2cch and 1o96 to form the best complex. In addition, the rationality of the results of network pharmacological screening was to further confirmed by cell experiments in vitro.

Conclusions

In this study, network pharmacology combined with molecular docking was used to elucidate the mechanism by which moorcroftiana alkaloids regulate lung cancer through multiple targets and channels. The results showed that oxymatrine, matrine, oxysophocarpine, sophocarpine, sophoridine and cytosine of SMA could form the best complex with 2W96, 2CCH and 1O96 target proteins, and to further verify the therapeutic effect of SMA in LC A549 cell, which also can provide a reliable reference basis that SMA were potential candidates in treating LC.

Declarations

Acknowledgments

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Author contributions

Designed the experiments: Peng Ji and Nian-Shou Zhao; Collection and assembly of data: Peng Ji and Nian-Shou Zhao; Data analysis and interpretation: Peng Ji and Nian-Shou Zhao ; Analyzed data and wrote the main manuscript text: Peng Ji and Nian-Shou Zhao; Final approval of the manuscript: all authors. All authors contributed to the article and approved the submitted version.

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Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

No competing financial and nonfinancial interests.

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Table 2

Table 2 is available in the Supplementary Files section.

Figures

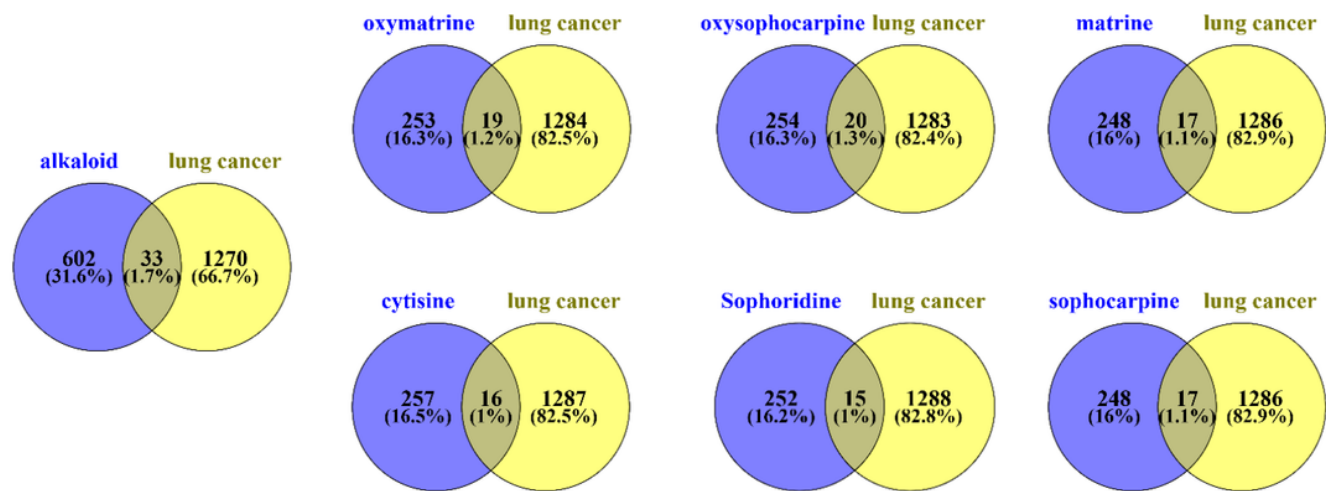


Figure 1

The intersection target of SMA and LC. The blue represents alkaloid target. The yellow represents LC disease target.

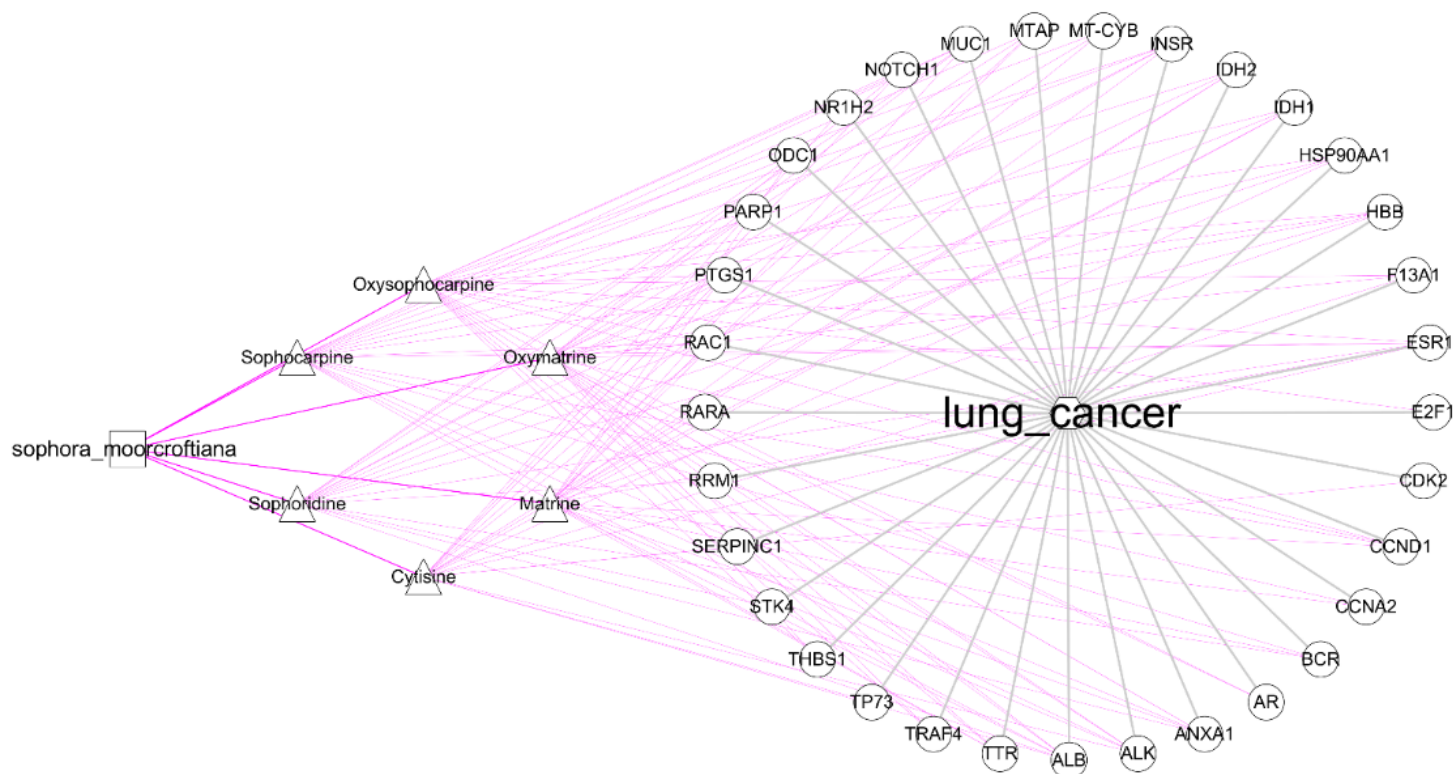


Figure 2

SMA and LC intersection targets network. The square represents SM. The triangle represents the SMA component. The circle represents the intersection targets. The hexagon represents LC disease.

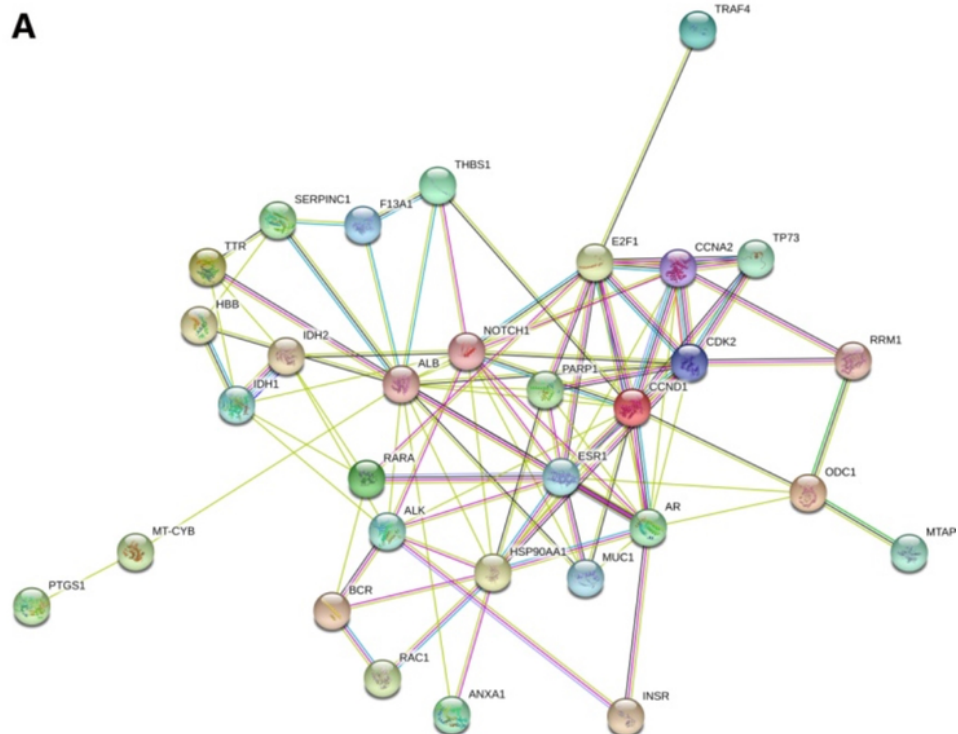
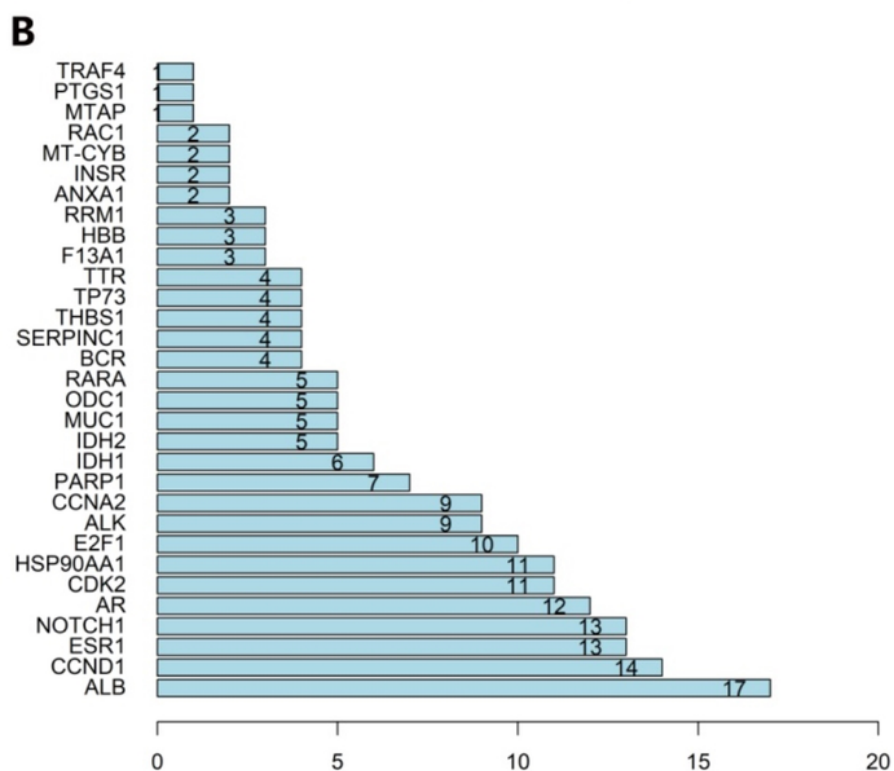


Figure 3

Protein and protein interaction network (PPI) and interaction network node value. (A) Interaction network of target proteins in SMA and LC. (B) Interaction network node value in SMA and LC. The Y-axis shows the target genes. The X-axis shows the gene count.

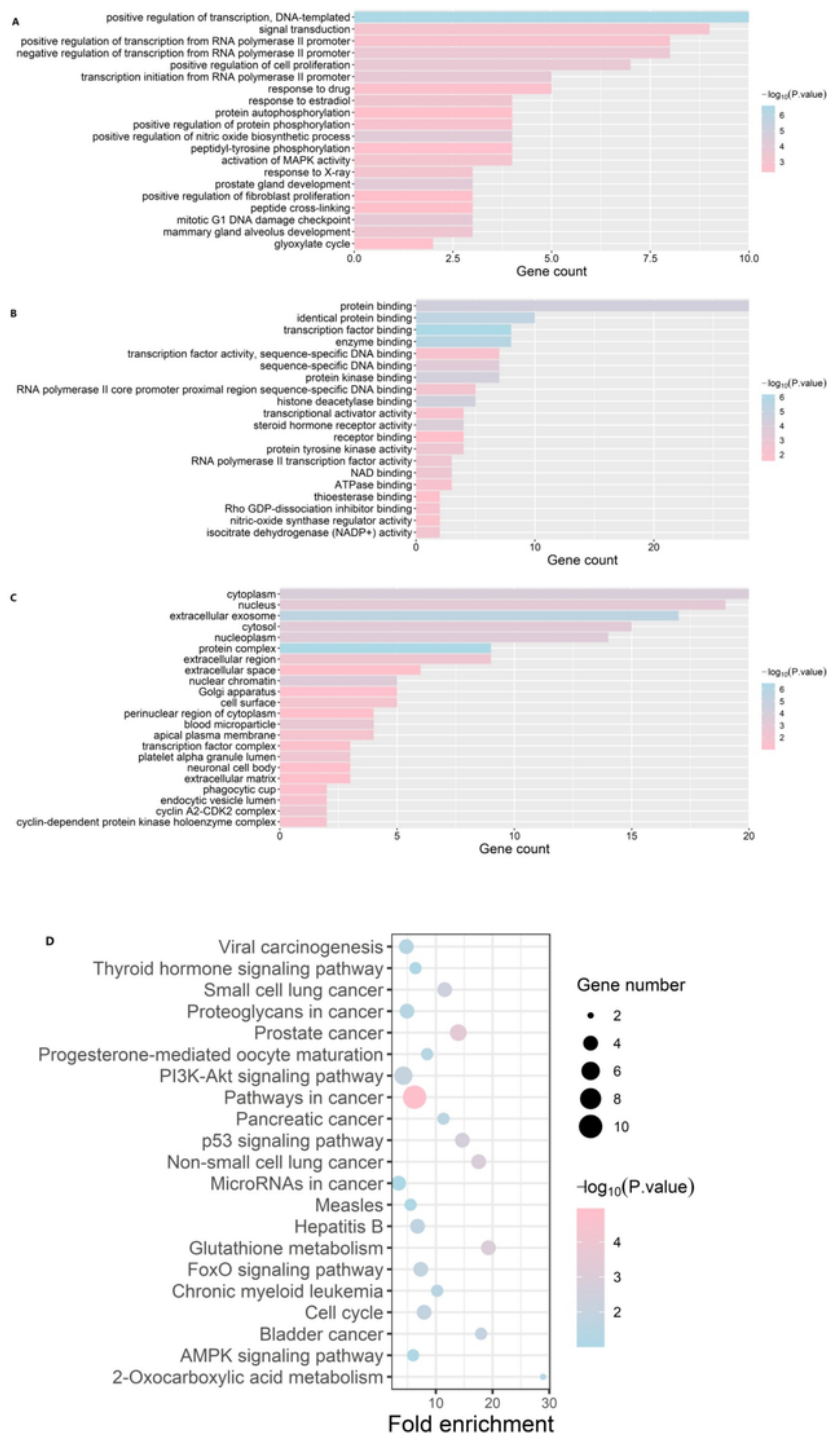


Figure 4

GO and KEGG enrichment analysis. A. Biological Process (BP) enriched. B. Molecular Function (MF) enriched. C. Cellular Component (CC) enriched. The Y-axis shows the significantly enriched GO categories of the target genes ($p\text{-value} < 0.01$). The X-axis indicates significantly enriched BP, MF, and CC count of targets. D. KEGG enrichment pathway. The Y-axis shows enriched KEGG pathways of the target genes. The X-axis displayed the rich factor ($p\text{-value} < 0.01$) and the dot's color reflects the different value ranges.

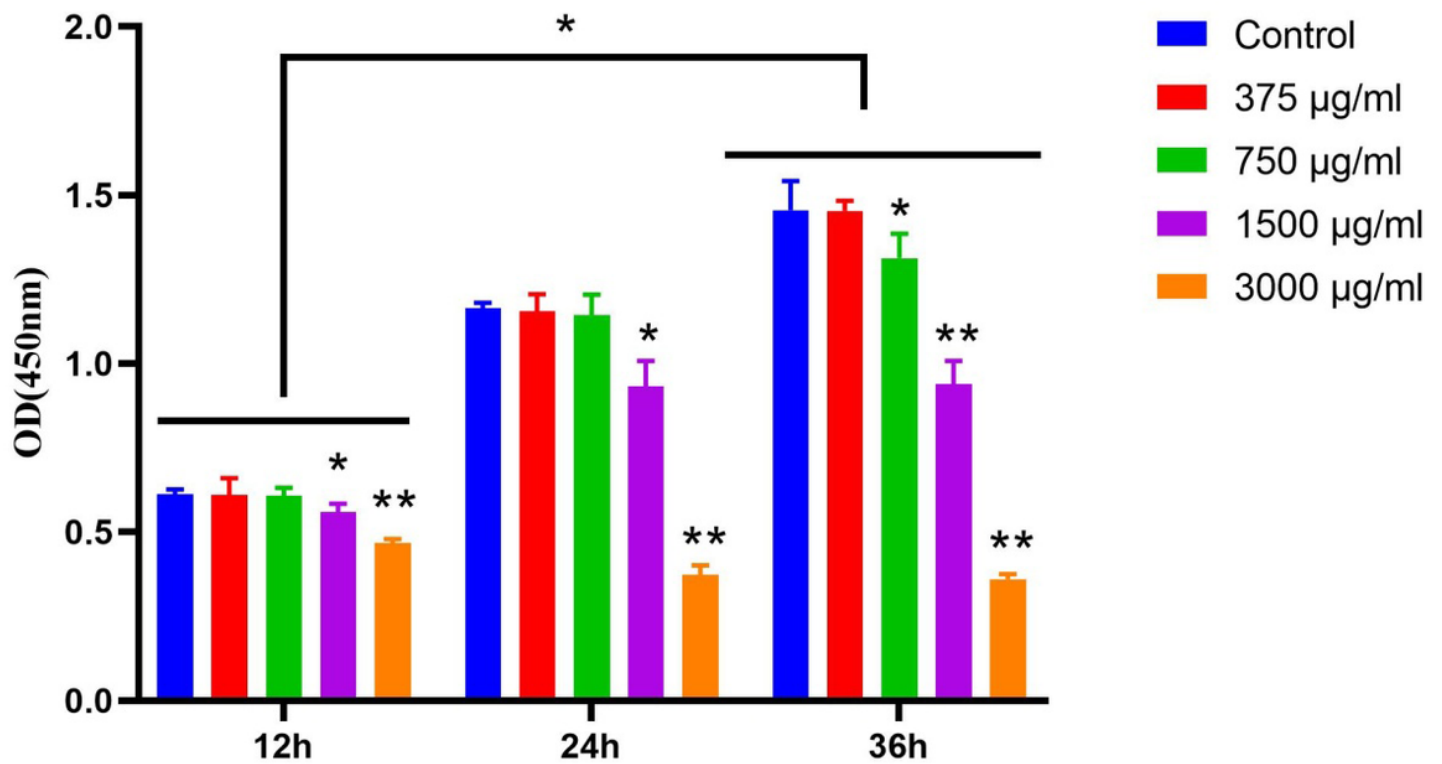


Figure 6

Changes in OD₄₅₀ value after treatment at different concentrations and different times. Data are shown as mean $\pm$ standard deviation. "*"p < 0.05, "**"p < 0.01 vs control.

Supplementary Files

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- [Table2.docx](#)