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Network Pharmacology and Molecular Docking Study on the Mechanism of the Therapeutic Effect of Strychni Semen in NSCLC

He Geng[1†], Yujie Xue[2†], Binghua Yan[1], Zhaoxue Lu[1], Hengjin Yang[1], Peng Li[1*] and Jundong Zhou[3,4*]

Abstract

Strychni Semen, characterized by its bitter taste and warm properties, has been confirmed to possess anti-tumor properties. However, the molecular mechanism of Strychni Semen in treating non-small cell lung cancer (NSCLC) needs further study. This study aimed to explore the molecular mechanism of Strychni Semen in treating NSCLC based on network pharmacology and molecular docking. The active components and targets of Strychni Semen were retrieved from the TCMSP, supplemented by the HERB database and the related literature. NSCLC-related targets were retrieved from the GeneCards, OMIM and DisGenet databases. The intersection targets of Strychni Semen in treating NSCLC were obtained via an online platform. The Protein-Protein Interaction (PPI) network was subsequently constructed to deeply analyse the interrelationship of the intersection targets via the String database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were carried out via the Metascape database. The interactive networks between Strychni Semen and NSCLC were constructed via Cytoscape 3.9.1. Molecular docking detected interactions between the key components and the core targets. The core targets were validated via GEO datasets. 21 active components and 67 targets were identified, with 47 associated with NSCLC. The key active components were Stigmasterol, IcarideA, 2-Hydroxymethylanthraquinone, (+)-catechin, (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one, (S)-Stylopine, Brucine and Isobrucine. The core targets were PTGS2, NR3C1, ESR1, CASP3 and PRKACA. Molecular docking revealed that these compounds undergo strong binding affinity with the core genes. GEO database indicated that PTGS2 was the most promising core target. In addition, Strychni Semen's effects on NSCLC involved mainly the Calcium pathway, the Estrogen pathway, and the cGMP-PKG and cAMP pathways. This study visually demonstrated the mechanism of the therapeutic effect of Strychni Semen in NSCLC through multiple components, targets and pathways which provides a basis for clinical treatment and further experimental research.

Keywords Strychni semen, NSCLC, Network pharmacology, Molecular docking, Signaling pathway

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Introduction

Non-small cell lung cancer (NSCLC) is the predominant epithelial lung cancer, accounting for approximately 85% of all lung cancer subtypes, in contrast to small-cell lung cancer (SCLC) [1, 2]. At the time of initial diagnosis, approximately one-third of NSCLC patients are at stage III, with a 5-year overall survival rate of only 20% [3, 4]. The advent of immune checkpoint inhibitors (ICIs) has represented a significant breakthrough in the treatment of stage III NSCLC, leading to a substantial prolongation of patient survival [2, 5–7]. However, owing to their toxicity and drug resistance, only 10-30% of patients benefit from ICIs [8–12]. Therefore, finding a drug that can effectively treat NSCLC with less toxicity has become the main focus of NSCLC research.

Traditional Chinese medicine is a valuable gift of nature and a natural treasure for the development of anti-tumor drugs. It has attracted increasing attention because of its high efficacy, good safety and low toxicity. Strychni Semen, the dry and mature seed of Strychnos nux-vomica L, belonging to the genus Strychnos of the family Loganiaceae, is bitter and warm in nature with effects including clearing collaterals, dispelling nodules, relieving pain, and reducing swelling [13]. It has been historically used to treat various diseases such as indigestion, neurological disorders, chronic rheumatism, urinary incontinence, and impotence [13]. Modern studies have proved that Strychni Semen has a certain therapeutic effect on many kinds of tumors, including NSCLC [14]. However, the mechanism by which Strychni Semen treats NSCLC remains unclear. Therefore, this study aimed to predict the molecular mechanism of Strychni Semen in treating NSCLC via network pharmacology and molecular docking, to provide a theoretical basis for clinical treatment and further experimental research.

Materials and Methods

The study strategy is illustrated in Fig. 1.

Screening of Strychni Semen Active Components and Targets

Using "Strychni Semen" as the search term, with Oral bioavailability (OB)≥20% and Drug-likeness (DL)≥0.1, the active components and targets were retrieved from the TCMSP (https:/tcmsp-e.com/tcmsp.php), supplemented by the HERB database (http://herb.ac.cn) and the related literature [13]. The selected targets were reintegrated into the UniProt database (https://www.uniprot.org), the species defined as "*homo sapiens*", and target protein-encoding gene information was standardized.

Screening of NSCLC-Related Targets

Using "Non-small cell lung carcinoma" as the keyword, human genes were obtained from the GeneCards (https://www.genecards.org), OMIM (https://www.omim.org) and DisGenet (https://www.disgenet.org) databases, and duplicate genes were subsequently eliminated to obtain NSCLC-related targets.

Construction of a "Drug-Component-Potential Target-Disease" Network and Screening of Key Active Components

A Venn diagram was generated on an online platform (https://www.bioinformatics.com.cn) to identify the intersecting genes and potential targets of Strychni Semen in treating NSCLC. These potential targets were subsequently utilized to construct a "drug-component-potential target-disease" network via Cytoscape 3.9.1. The degree value of each node in the network was calculated via CentiScape2.2, and key active components were identified for molecular docking.

Construction of a PPI Network and Screening of Core Targets

The intersecting genes were uploaded to the STRING database (https://string-db.org), the species defined as "*homo sapiens*", with medium confidence (0.4). The PPI network was generated and divided into three clusters via k-means. Visualized via Cytoscape 3.9.1, the hub genes were determined by the Maximal Clique Centrality (MCC), Closeness, Degree and Radiality respectively via the Cytohubba plugin. A Venn diagram was generated based on the intersecting genes to prepare for molecular docking.

GO and KEGG Enrichment Analysis

The effective targets within the PPI network were uploaded to Metascape data (https://metascape.org), the species defined as "*homo sapiens*" and the P value<0.01 set as the criteria for enrichment analysis of GO and KEGG pathways. The top 10 GO terms and the top 20 KEGG pathways with significant differences were selected for visualization.

Construction of a "Drug-Component-Potential Target-Pathway-Disease" Network

The top 20 KEGG pathways were collected and uploaded to Cytoscape 3.9.1 together with drug components and potential targets in order to construct a "drug-component-potential target-pathway-disease" network.

Molecular Docking

The 2D structures of the key active components (ligands) were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov), saved in SDF format, then converted to 3D by Chem3D 22.2 with bond energy minimization and angle adjustment, and exported in mol format. The 3D structures of the core target (receptor) were from RCSB

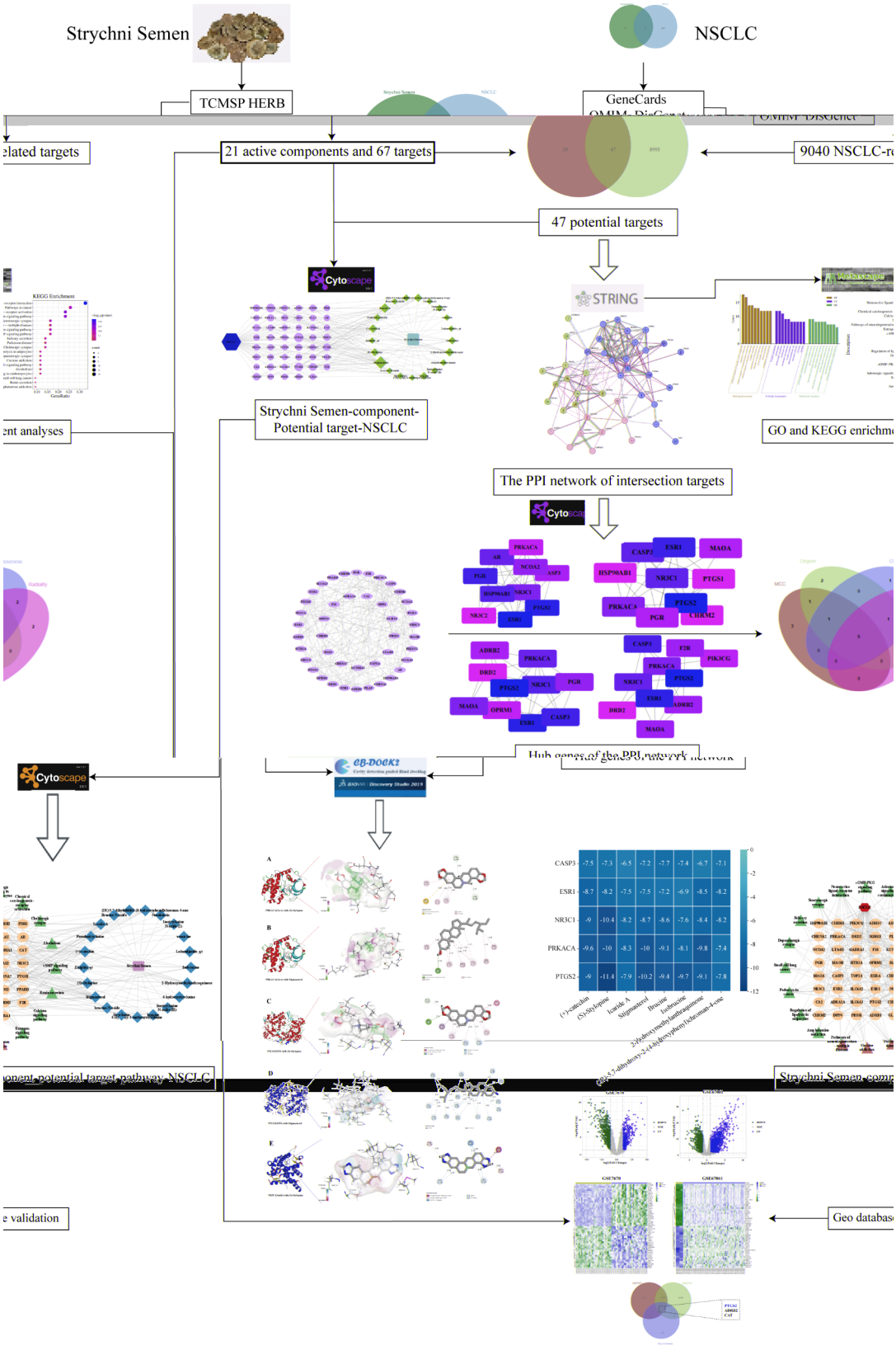


Fig. 1 Flowchart of the study

Table 1 Active components of Strychni Semen

Mol/Herb ID	Component name	Oral bioavailability (OB) (%)	Drug-likeness (DL)
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
MOL001476	(S)-Stylopine	51.15	0.85
MOL003410	Ziziphin_qt	66.95	0.62
MOL003411	Icaride A	48.74	0.43
MOL003413	Isostrychnine N-oxide (I)	35.45	0.8
MOL003414	Isostrychnine N-oxide (II)	37.33	0.8
MOL003418	Lokundjoside_qt	32.82	0.76
MOL003422	Novobiocin	21.89	0.68
MOL003425	Pseudostrychnine	24.63	0.53
MOL003427	2-Hydroxymethylanthraquinone	21.07	0.18
MOL003432	Vomicine	47.56	0.65
MOL003433	Brucine-N-oxide	49.17	0.38
MOL003434	4-Hydroxy-3-methoxystrychnine	20.93	0.79
MOL003436	Isobrucine	33.58	0.8
MOL003438	4-hydroxystrychnine	28.34	0.52
MOL003440	Brucine N-oxide	52.63	0.38
MOL000449	Stigmasterol	43.83	0.76
MOL000492	(+)-catechin	54.83	0.24
MOL003018	SCG	23.59	0.36
MOL003435	Brucine	7.61	0.41
HBIN045003	Strychnine	-	-

PDB (https://www.rcsb.org) in PDB format. Ligands and receptors underwent docking on CB-Dock2 (https://cadd.labshare.cn/cb-dock2), and results were analyzed and visualized using Discovery Studio 2019.

GEO Database Validation

The "limma" R package was used to analyze the differential gene expression of two NSCLC datasets GSE7670 and GSE67061 in GEO database (https://www.ncbi.nlm.nih.gov/geo/). The significance threshold criterion was $|\log FC| > 1$, and the adjusted p value was < 0.05. Volcano plots and heatmaps were generated using the "ggplot2" and "pheatmap" R packages, respectively. The Venn diagram was utilized to identify the common targets among Strychni Semen potential targets and the two NSCLC datasets, GSE7670 and GSE67061.

Results

Screening of Strychni Semen Active Components and Targets

A total of 62 components of Strychni Semen were obtained from the TCMSP database. According to OB≥20% and DL≥0.1, non-target components were excluded, leaving 19 effective components. Brucine and Strychnine, key bioactive and toxic components, were added despite not meeting criteria. Consequently, a total of 21 active components were selected, with Strychnine being supplemented with the HERB database, as shown in Table 1. The targets of these active components were transformed, corrected, and deduplicated via the UniProt database, resulting in a total of 67 targets.

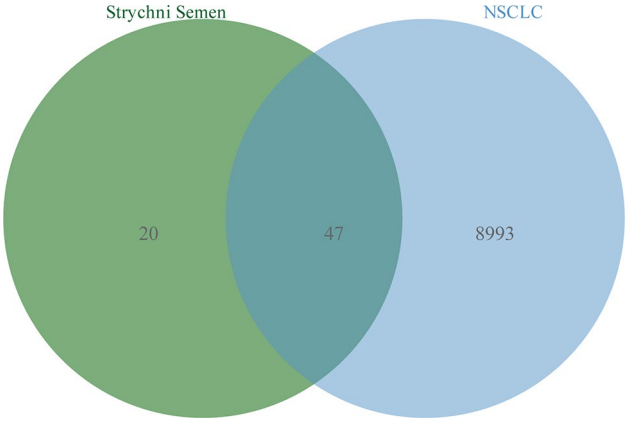


Fig. 2 The Venn diagram of Strychni Semen and NSCLC intersecting targets

Screening of NSCLC-Related Targets

A total of 7279, 1186 and 3926 NSCLC related genes were respectively retrieved from the GeneCards, OMIM and DisGeNET databases, respectively, and a total of 9040 targets were obtained after eliminating duplicate genes. The corresponding targets of Strychni Semen active components were intersected with NSCLC-related targets and 47 potential targets were obtained via a Venn diagram (Fig. 2).

The "Strychni Semen-component-Potential target-NSCLC" network was constructed via Cytoscape 3.9.1,

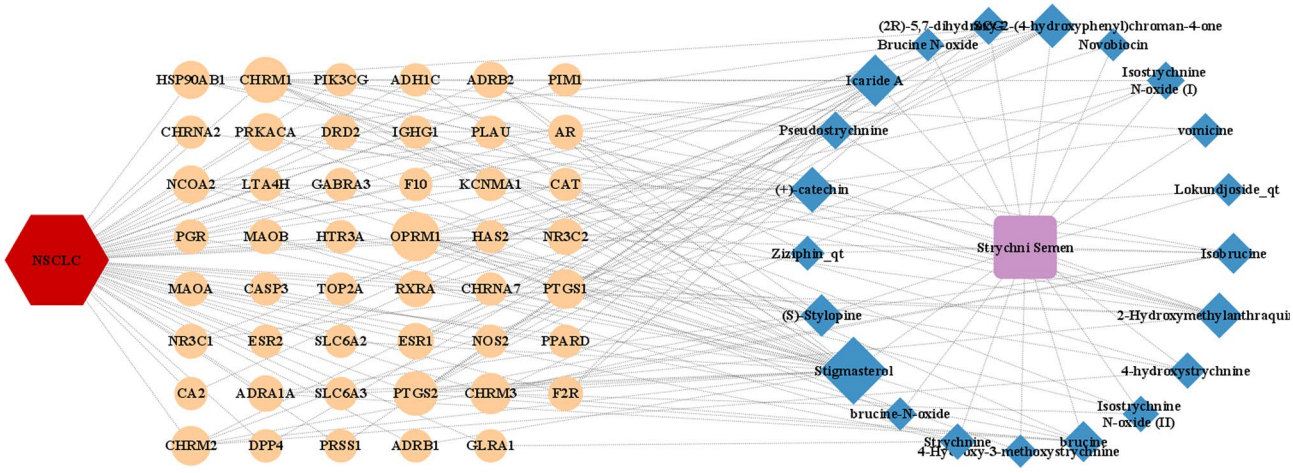


Fig. 3 The network of Strychni Semen-component-Potential target-NSCLC. The purple square represents Strychni Semen, blue diamonds represent active components, brown circles represent intersection targets, and red hexagon represents NSCLC.The node size stand for the size of the degree. Node size is proportional to its degree

Table 2 The key active components of Strychni Semen

Component name	Degree
Stigmasterol	24
Icaride A	14
2-Hydroxymethylanthraquinone	10
(+)-catechin	10
(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chRoman-4-one	9
(S)-Stylopine	8
Isobrucine	7
Brucine	7

The median degree value was 5

which comprise of 70 nodes and 179 edges (Fig. 3). The Degree value of node in the network was calculated via CentiScape2.2, and 8 key active components were screened under conditions where the node degree value exceeded the median value within the network, as shown in Table 2.

Construction of a PPI Network and Screening of Core Targets

The 47 potential targets were uploaded to the String database to construct the PPI network (Fig. 4). Three free nodes and one unidentified node were removed, resulting in a total of 43 nodes and 179 edges in the PPI network after the information was imported into Cytoscape 3.9.1 (Fig. 5A). Using the Cytohubba plugin, the top 10 genes for the treatment of NSCLC with Strychni Semen were screened using the MCC, Degree, Closeness, and Radiality algorithms, respectively. Red, orange, and yellow represent a gradual decrease in Score from largest to smallest, with darker colors indicating a more central position (Fig. 5B-E). Further, a Venn diagram was generated to identify five core targets: PTGS2(5f19), NR3C1(4udd), ESR1(1xpc), CASP3(1nme) and PRKACA(3ovv). These genes may be potential targets for the treatment of NSCLC with Strychni Semen, constituting the core hubs of the PPI network (Fig. 5F).

GO and KEGG Enrichment Analysis

The enrichment analysis of 43 effective targets was conducted using the Metascape database with $p<0.01$ for GO enrichment analysis generating 362 Biological Processes (BP), 34 Cellular Components (CC) and 73 Molecular Functions (MF). The top 10 BP, CC, and MF terms are plotted as bar graphs (Fig. 6A). Additionally, KEGG pathway analysis yielded a total of 53 pathways, and bubble plots illustrating the top 20 enriched pathways were created (Fig. 6B). These top 20 KEGG pathways, along with their components and targets were then imported into Cytoscape3.9.1 to construct a "drug-component-potential target-pathway-disease" network (Fig. 7).

Molecular Docking

The 8 key active components of Strychni Semen were sequentially docked with the 5 core targets, resulting in a binding energy < -5.0 kcal/mol (Fig. 8). A binding energy < -5.0 kcal/mol indicates certain binding activity, whereas a binding energy < -7.0 kcal/mol indicates strong binding activity [15]. In other words, the lower the binding energy, the more stable the binding conformation. The docking results with binding energy ≤ -10.0 kcal/mol were visualized (Fig. 9A-E).

GEO Database Validation

The two NSCLC datasets GSE7670 and GSE67061 in the GEO database were differentially analyzed to obtain potential targets using the "limma" R package. The number of Differentially Expressed Genes (DEGs) was represented using volcano plots, where red dots indicating

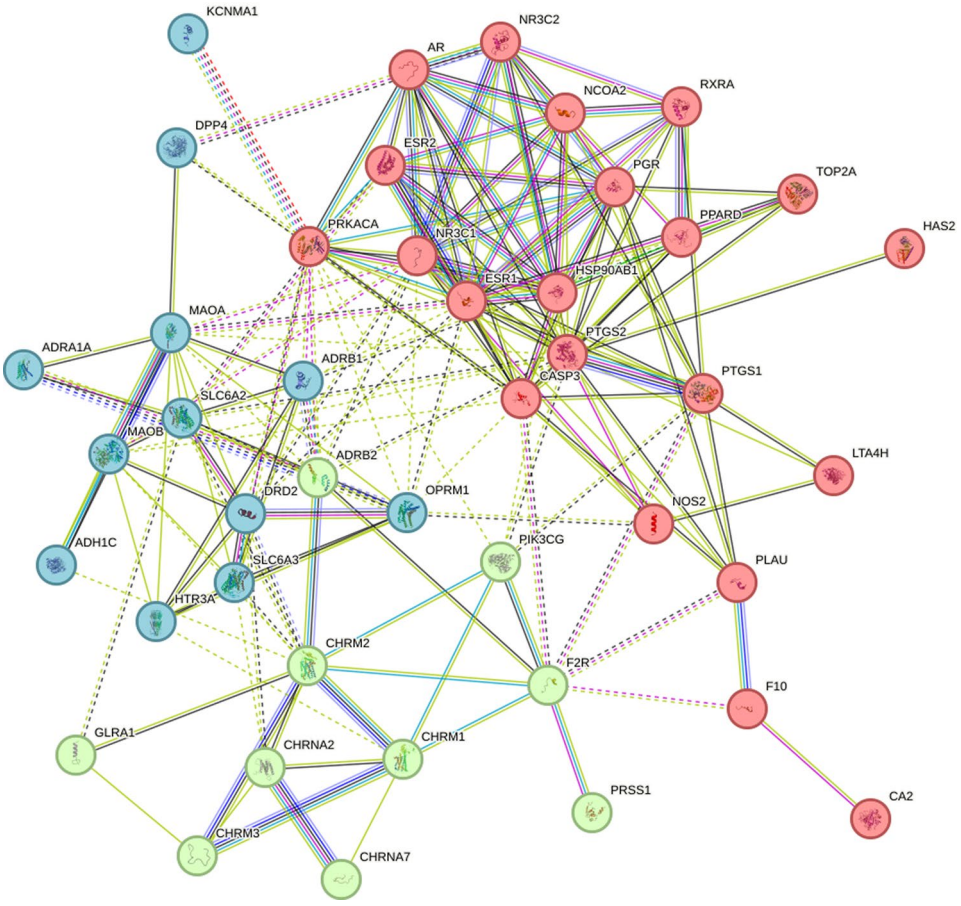


Fig. 4 The PPI network of intersection targets

up-regulated genes and blue dots indicating down-regulated genes. The distribution of DEGs was visualized in the heatmap, with 25 up-regulated and 25 down-regulated genes detailed in different groups. Finally, the common targets between the drug targets and the two datasets were screened, thus identifying the 3 core genes. Among them, PTGS2 was identified as a core target of Strychni Semen's effect on NSCLC through network pharmacology (Fig. 10).

Discussion

NSCLC is the leading cause of cancer death worldwide, with approximately 70% of patients being diagnosed with advanced or distant metastases and unable to undergo radical surgery [16]. Consequently, most patients are treated with radiation therapy, immunotherapy, or medication. However, drug toxicities such as rash, diarrhea, nausea, vomiting, liver dysfunction, and cellular immunodeficiency often result in dose reduction or discontinuation [17]. Traditional Chinese medicine is an effective treatment method for NSCLC that not only reduces toxicity and enhances effectiveness but also relieves symptoms and improves quality of life to a certain extent. It directly inhibits the growth, proliferation, invasion and migration of tumor cells [18]. Strychni Semen has a long history of treating tumors and was first recorded in the Compendium of Materia Medica for its "eliminating mass" effect. However, its therapeutic effect and mechanism in NSCLC remain unclear.

In this study, we constructed the "Strychni Semen-component-Potential target-NSCLC" network using 21 active components and 47 intersecting targets. A total of 8 key active components were screened: Stigmasterol, Icaride A, 2-Hydroxymethylanthraquinone, (+)-catechin, (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)-chroman-4-one, (S)-Stylopine, Brucine and Isobrucine. Among these components, Stigmasterol has the most intersecting targets with NSCLC. Study has shown that stigmasterol can activate pro-apoptotic protein signals and stimulate the cleavage of caspase-3, caspase-9, cytochrome c, BAK and BAX in a dose-dependent manner to induce apoptosis in ovarian cancer [19]. Furthermore, Stigmasterol combined with Sorafenib can promote caspase-3 activity while down-regulating the levels of Bcl2, NF-kB, Ki-67, VGEF-A and VEGFR-2, thus inhibiting angiogenesis and promoting apoptosis in breast cancer

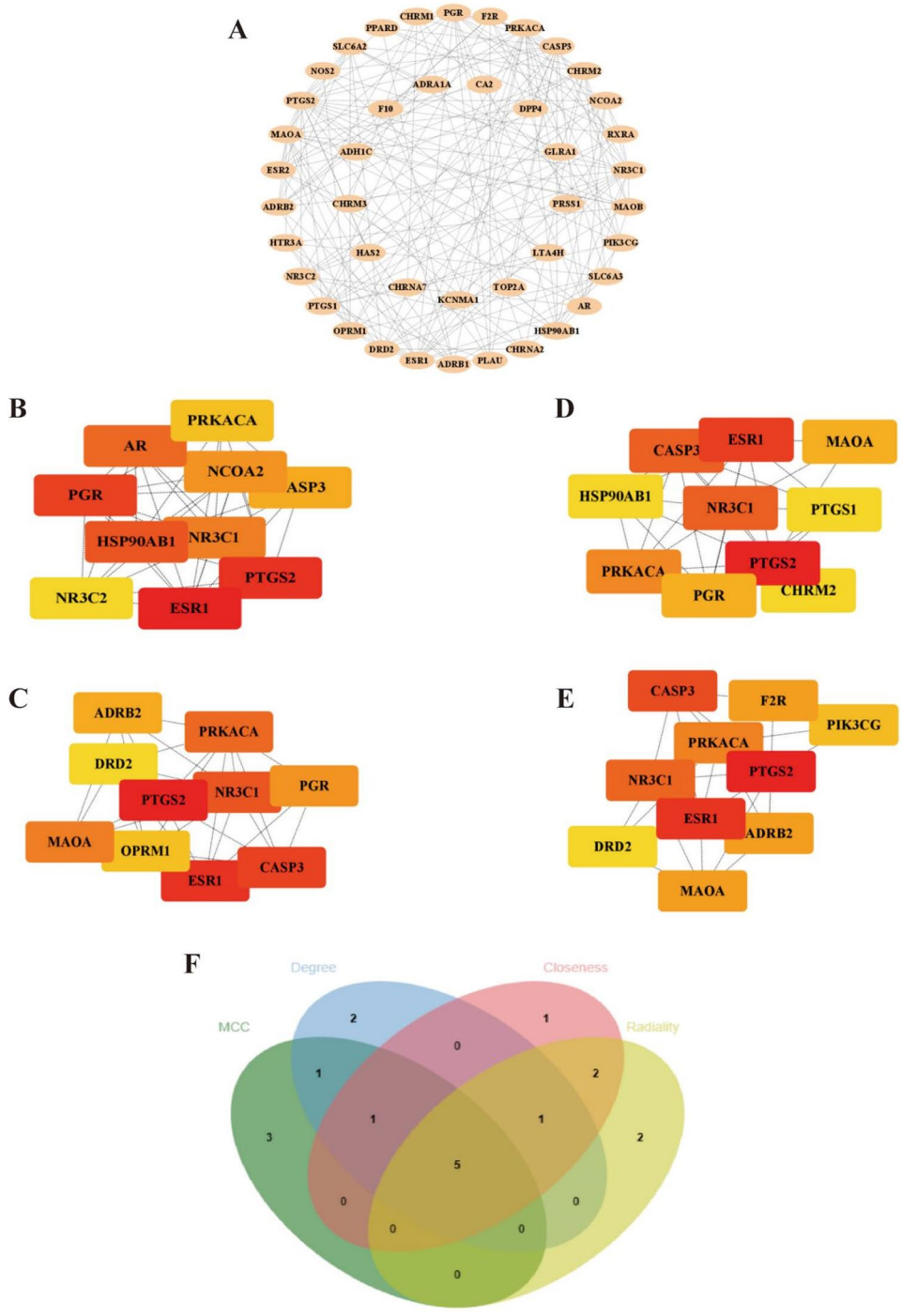
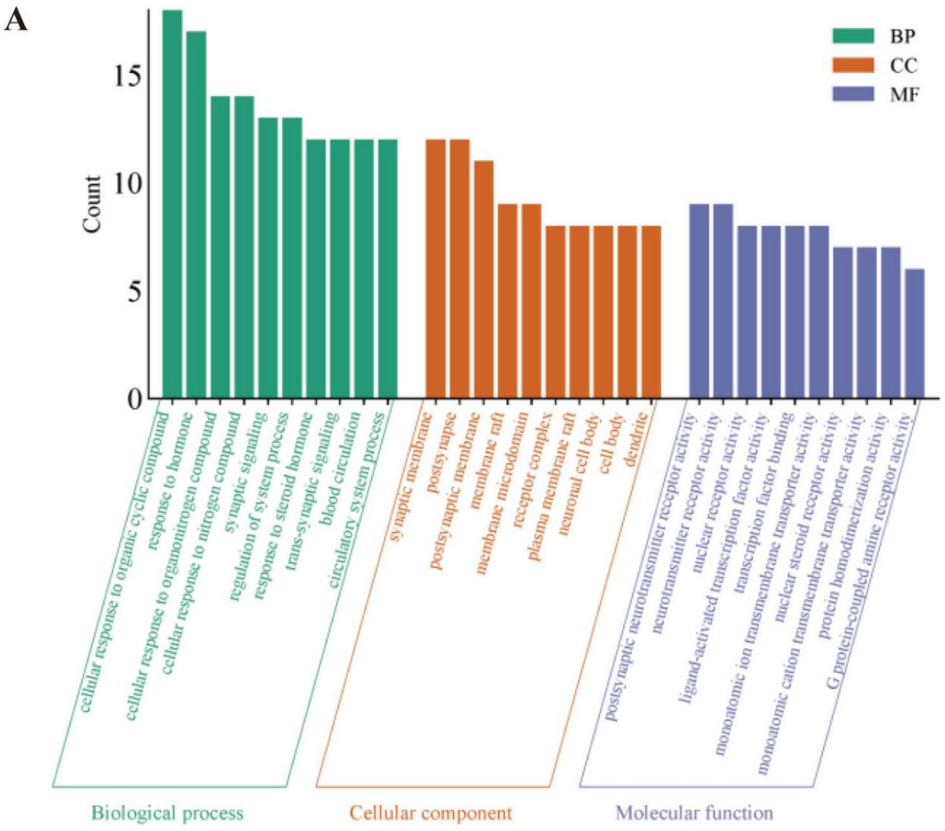


Fig. 5 Hub genes of the PPI network .(**A**) The PPI network was reconstructed by Cytoscape 3.9.1. (**B-E**) Top 10 genes in network ranked by MCC, Closeness, Degree and Radiality algorithms, respectively. (**F**)The venn diagram of MCC, Degree, Closeness and Radiality

[20]. In addition, Stigmasterol can down-regulate TNF-α and inhibit VEGFR-2, including that of phosphorylated Akt, Src, PCL, Akt and FAK, consequently disrupting tumor angiogenesis and reducing the growth of cholangiocarcinoma [21]. Notably, Stigmasterol can enhance the sensitivity of endometrial cancer cells to chemotherapy by down-regulating the Nrf2 signaling [22]. (S)-Stylopine is a protoberberine-type alkaloid with unique chemical structure and potential biological activities [23, 24]. Research has shown that (S)-Stylopine exhibits excellent affinity and highly specific interaction with CK2, making it an effective inhibitor of CK2 and demonstrating



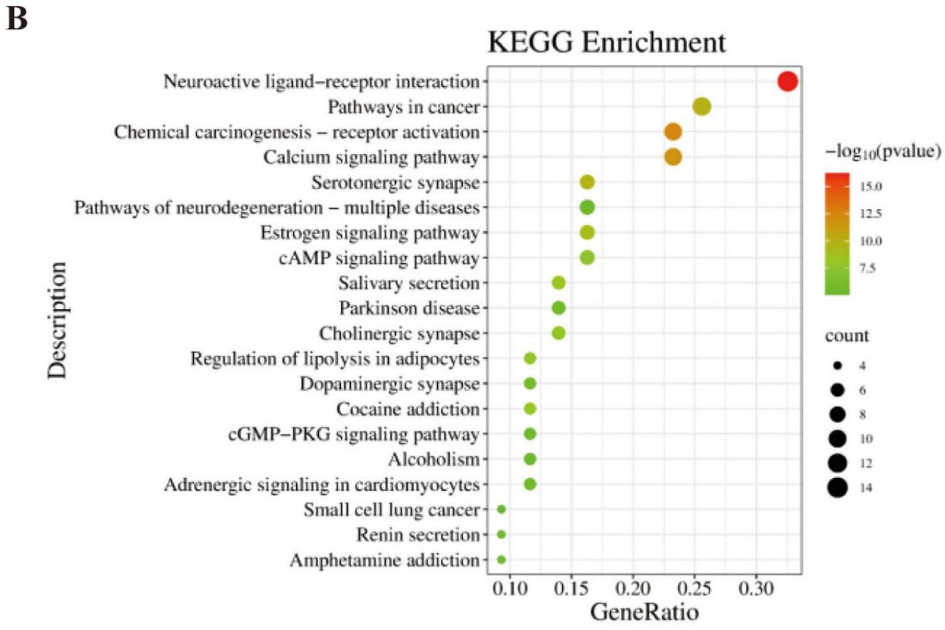


Fig. 6 GO and KEGG enrichment analyses. (**A**) GO enrichment analysis. The top 10 terms of each part were displayed with a bar chart. (**B**) KEGG pathway enrichment analysis. The top 20 pathways were displayed in a bubble chart.The bubble size indicates the number of genes enriched by the pathway; the redder the bubble color, the greater the corrected *P* value and the greater the enrichment significance

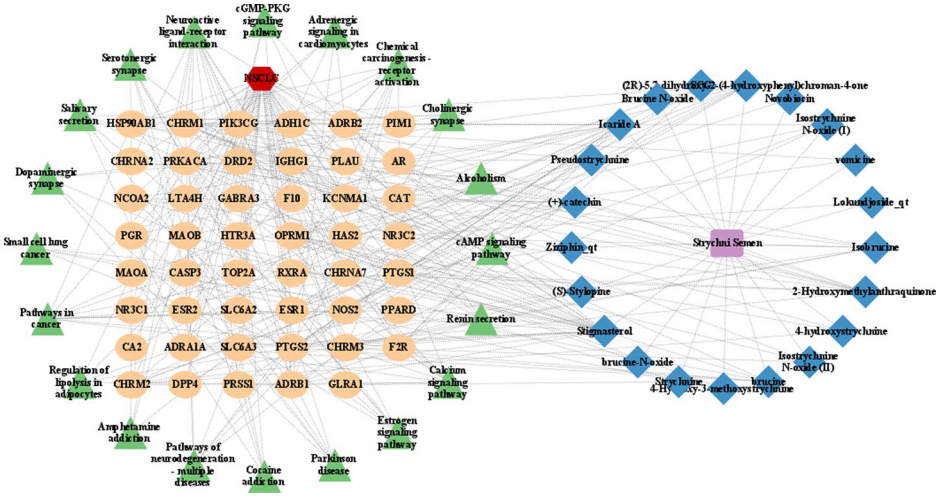


Fig. 7 The network of Strychni Semen-component-potential target-pathway-NSCLC

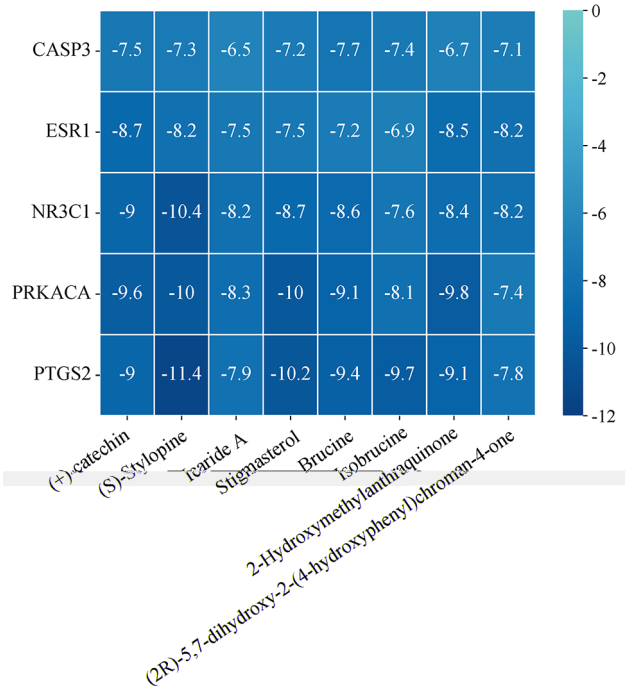


Fig. 8 Binding energy heatmap of molecular docking. The darker the color represents, the lower the binding energy

immense potential in the field of cancer treatment [25]. In particular, coptisine, an oxidized form of (S)-Stylopine, has been demonstrated to suppress cell viability and invasion of NSCLC cells while contributing to apoptosis [24, 26]. The mechanism involves inducing cell cycle arrest at G2/M phase and reactive oxygen species-dependent mitochondria-mediated apoptosis in NSCLC cells [27]. (+)-catechin is an important flavonoid with extensively studied anti-tumor properties. It induces the expression of the cyclin kinase inhibitor p21 and inhibits the expression of cyclin E1. Additionally, it is cytotoxic to A549 cells in a dose-dependent manner, resulting in cell cycle arrest [28]. Furthermore, (+)-catechin regulates the production of pro-inflammatory factors such as nitric oxide, VEGF, IL-2, TIMP-1 and other pro- and anti-angiogenic factors. This consequently inhibits tumor-specific angiogenesis [29]. Promisingly, (+)-catechin specifically targets the Pgp, M RP1, MRP2, MDR1, LRP and BCRP proteins affecting drug resistance in various types of tumor cells [30]. Brucine is one of the main active and toxic components of Strychni Semen with favorable anti-tumor properties at safe doses. It can regulate bone metastasis-related factors such as MMP-2, CXCR4, RANKL and OPG thereby inhibiting the bone metastasis of breast cancer [31]. In a nude mouse model of bone metastasis from breast cancer, brucine can down-regulate VEGF expression consequently inhibiting angiogenesis, growth and bone metastasis of the tumor [32]. Brucine either individually or in combination with gemcitabine can inhibit p65 expression, resulting in death of MCF7 cells in G2 phase [33]. As expected, Bru-TD-Lip which are highly capable of encapsulating brucine and stable, can significantly inhibit the proliferation, migration and invasion of TNBC cells, consequently effectively targeting and inhibiting subcutaneous mammary carcinogenesis in female nude mice [34]. Strychnine is another main component of Strychni Semen. Although it was not considered as one of the key active components in the present study, previous research has demonstrated its anti-tumor properties at safe doses. it can down-regulate VEGF, TNF-α, and TGF-β levels, and inhibit inflammation-induced angiogenesis, consequently preventing tumor growth and metastasis [35]. Additionally, it can trigger Wnt/β-catenin, up-regulate the expression of DKK1 and APC, and down-regulate the expression of PITX2, β-catenin, c-Myc and MMP2, thereby leading to targeted inhibition of colon cancer growth [36].

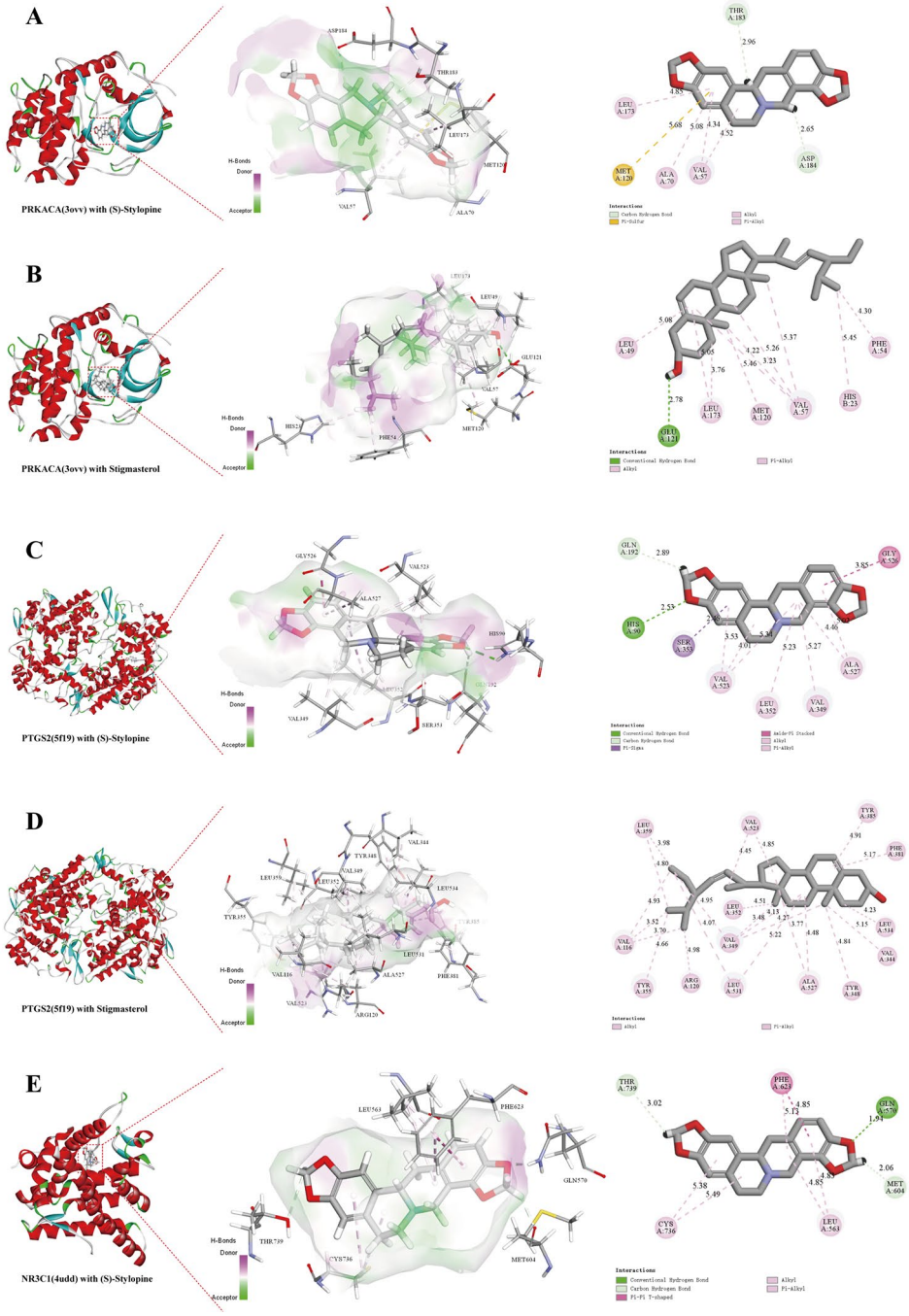


Fig. 9 Molecular docking with binding energy ≤ -10 kcal/mol. (**A**) PRKACA(3ovv) with (S)-Stylopine. (**B**) PRKACA(3ovv) with Stigmasterol. (**C**) PTGS2(5f19) with (S)-Stylopine. (**D**) PTGS2(5f19) with Stigmasterol. (**E**) NR3C1(4udd) with (S)-Stylopine

In this study, a total of 5 hub genes were identified through the PPI network: PTGS2, NR3C1, ESR1, CASP3 and PRKACA. These genes may serve as potential targets for Strychni Semen in treating NSCLC. For example, PTGS2 encodes cyclocsynthase 2 (Cox-2), which is the rate-regulating enzyme for the conversion of arachidonic acid into prostaglandins. Downregulation of Cox-2 expression can increase the the radiosensitivity of lung cancer cells [37], thereby promoting their apoptosis [38]. Furthermore, Cox-2 inhibitors have been shown to interfere with tumorigenesis, providing a new idea for intervention in the development and metastasis of lung cancer [39]. Another gene identified is NR3C1 which encodes GR, a member of the steroid hormone receptor superfamily. GR can protect itself from ubiquitin-mediated proteasome degradation through interaction

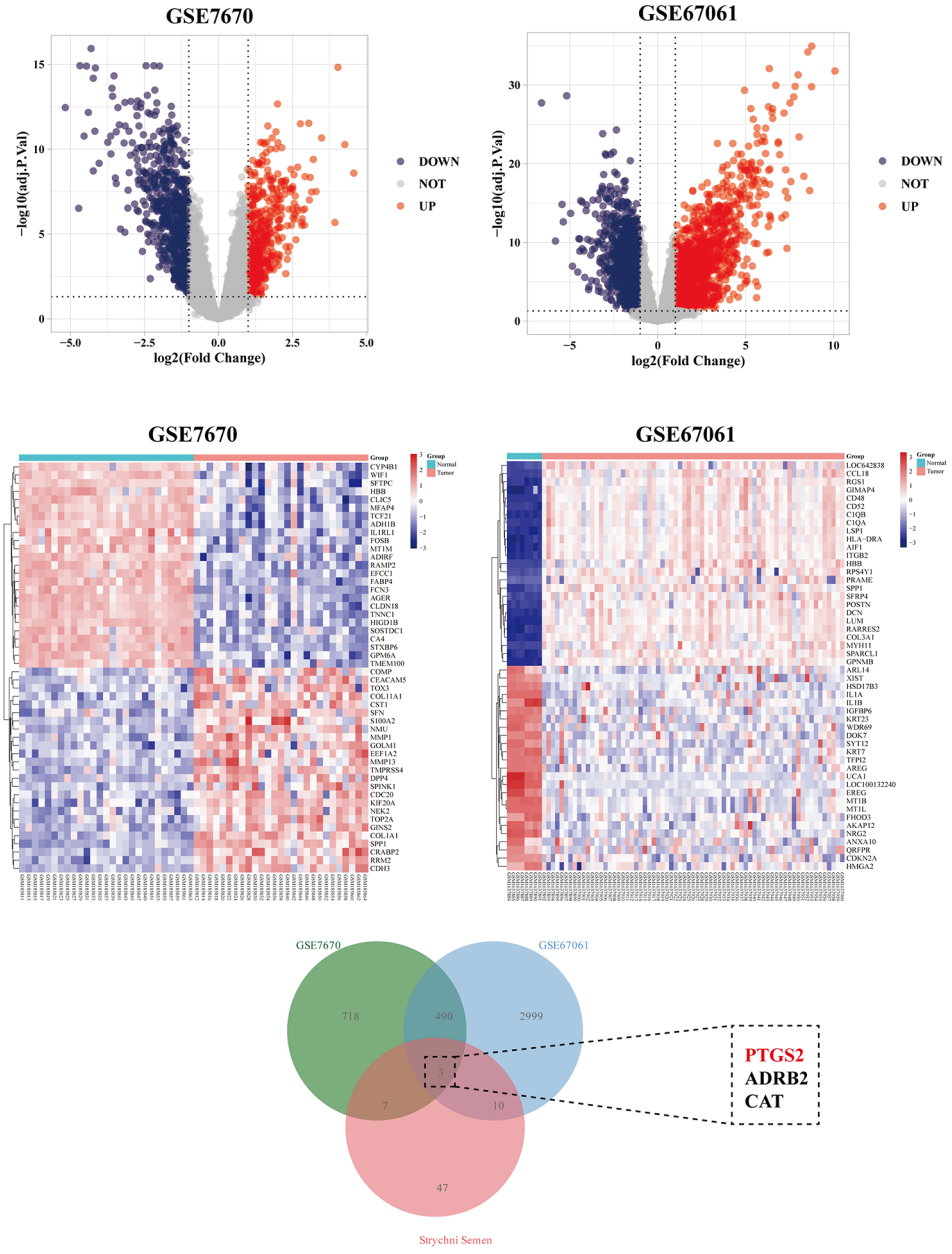


Fig. 10 Validation of core targets in Strychni Semen from datasets of GEO (GSE7670 and GSE67061)

with DEX-stimulated phosphorylation of JNK, and plays a role as a tumor suppressor in NSCLC cells [40]. It can also mediate DEX to up-regulates the protein expression of the CDK inhibitors p21 and p27 as well as CDKs 4 and 6 and induce the over-phosphorylation of Rb, leading to cell cycle arrest and irreversible senescence, and consequently inhibiting the growth of A549 cells [41]. ESR1 encodes Estrogen receptors (ERs), which are also a member of the steroid hormone receptor superfamily. ERs can adjust the EGFR, Notch1, and GSK3β/β-Catenin pathways, inducing cell proliferation, migration, invasion, and apoptosis escape, thereby promoting the progression of NSCLC [42]. They can also activate the CCL2/CCR2 axis and promote macrophage infiltration, M2 polarization and MMP9 production, thereby enhancing the invasiveness of NSCLC cells [43]. They have also been reported to engage in crosstalk with important therapeutic targets for NSCLCs, including EGFR, FGFR, and IGF1R [42]. CASP3 encodes Caspase-3, which is a member of the cysteine aspartate protease family. Caspase-3 can activate the DDR and downstream Cox-2/PGE2 axes, thus inducing tumor reproliferation during radiotherapy in NSCLC [44]. It can also activate the ERK pathway in a protease-independent manner, consequently promoting

lung metastasis and migration [45]. PRKACA encodes PKA-Cα, which is the catalytic subunit α of cAMP-dependent protein kinase A. Hypoxia can increase the expression of PKA-Cα, and the knockdown of PKA-Cα can prevent hypoxia-induced migration and invasion of lung cancer cells [46]. As a downstream effector of PGA-Cα, CREB can inhibit lipid peroxidation by binding to the promoter region of GPX4, and knockdown of CREB can inhibit the transcription of GPX4, thereby causing iron death in LUAD cells [47].

GO enrichment analysis revealed that Strychni Semen is involved mainly in biological processes such as the cellular response to organic cyclic compound, response to hormone, cellular response to organonitrogen compound, cellular response to nitrogen compound, synaptic signaling. Moreover, it may also affect some cell components and molecular functions in the treatment of NSCLC, including the synaptic membrane, postsynapse, postsynaptic membrane, membrane raft, membrane microdomain, postsynaptic neurotransmitter receptor activity, neurotransmitter receptor activity, nuclear receptor activity, ligand-activated transcription factor activity, and transcription factor binding. Synapses play an important role in the development of NSCLC. A recent study showed that DNMTi treatment of cancer cells enhances immune synapse formation between ex vivo expanded allogeneic γδ T cells and NSCLC cells and potentiates antitumor immunity by γδ T cells. Moreover, combined treatment of DNMTi and γδ T cells prolongs survival in mice bearing H1299 xenografts [48]. KEGG pathway enrichment analysis revealed that the treatment of Strychni Semen for NSCLC involves pathways such as the calcium pathway, estrogen pathway, cGMP-PKG pathway and cAMP pathway. The Calcium pathway can regulate I-type voltage-dependent Ca^{2+} channels by activating the Ca^{2+}-dependent protein kinase CaM to increase Ca^{2+} and Fe^{2+} uptake, leading to ROS accumulation, GSH depletion, and lipid peroxidation, thereby activating iron death and inhibiting NSCLC cell growth and migration [49]. It can also activate Ca2+-dependent protein kinase Iγ (CaMKII γ) and enhance the stem-like properties and tumorigenicity of lung cancer cells in an Akt- and β-catenin-dependent manner [50]. Estrogen signaling can adjust the EGFR, Notch1, GSK3β/β-Catenin pathways, inducing cell proliferation, migration, invasion, and apoptosis escape, thereby promoting the progression of NSCLC [42]. It can also activate the CCL2/CCR2 axis and promote macrophage infiltration, M2 polarization and MMP9 production, thereby enhancing the invasiveness of NSCLC cells [43]. In the cGMP-PKG pathway, cGMP-activated PKG can down-regulate the expression of β-catenin, and inhibit the MAPK pathway, consequently inducing the apoptosis of NSCLC cells [51]. In addition, it can promote the phosphorylation of cAMP-responsive element binding protein (CREB) and maintain the high expression levels of c-IAP1, Livin, Survivin and Mcl-1, therefore, inhibiting PKG-Iα kinase activity can enhance the pro-apoptotic effects of cisplatin on NSCLC cells [52]. The cAMP pathway can regulate LKB1 through CREB to affect the activity of the LncRNA LNC00473, and subsequently induce multidrug resistance in NSCLC [53]. Moreover, it can also mediate MHY4571 to inhibit PKA activation, prevent the phosphorylation of CREB, and consequently reducing the viability of NSCLC cells and promoting caspase 3-dependent cell apoptosis [54].

Molecular docking showed that Stigmasterol, Icaride A, 2-Hydroxymethylanthraquinone, (+)-catechin, (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-one, (S)-Stylopine, Brucine and Isobrucine have the good binding ability with PTGS2, NR3C1, ESR1, CASP3 and PRKACA, which indicated that the binding of these active components and core targets play an important role in the treatment of NSCLC by Strychni Semen. Furthermore, our research reveals that two NSCLC datasets in the GEO database overlap with Strychni Semen-related targets to yield PTGS2, further reinforcing the notion that PTGS2 plays a key role in the treatment of NSCLC with Strychni Semen.

Conclusion

This study determined the therapeutic effect of Strychni Semen in NSCLC through through multiple components, targets and pathways, providing a preliminary theoretical basis for the development of new drugs and clinical trials for NSCLC. At present, we only used bioinformatics methods to study the molecular mechanism of Strychni Semen in the treatment of NSCLC. Therefore, there are still limitations. First, the database data are from retrospective and predictive data, which may lack unidentified and unrecorded data. Second, the results of this study need to be further verified and explored by pharmacodynamics and experiments. In summary, we believe that this subject has great research potential and application value.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author Contributions

The authors declare that all data were generated in-house and that no paper mill was used. HG and YJ-X are co-first authors. HG and YJ-X performed the study, collected important experimental data and wrote the manuscript.

BH-Y, ZX-L and HJ-Y provided software and technical assistance. PL and JD-Z conceived and designed the experiments. All authors have read and approved the manuscript.

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Data Availability
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
Not applicable.

Competing Interests
The authors declare no competing interests.

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