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Research

Exploring the bioactive compounds derived from Plumula Nelumbinis and potential targets for the treatment of non-small cell lung cancer: A network pharmacology study

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Abstract:
Background: Plumula Nelumbinis (PN), derived from the mature green embryo of the Nelumbonaceae, has been widely used as an anti-inflammatory and antioxidant health product. Extensive evidence demonstrated that PN and its extracts might also have anti-cancer effects. In this study, we aimed to explore the potential physiological effects and molecular mechanisms of PN against non-small cell lung cancer (NSCLC).
Methods: We performed a network pharmacology strategy to explore molecular mechanisms of PN against NSCLC. The TCMSP databases and literature research were used to collect the active compounds. The compounds-target genes were obtained from the TCMSP, PubChem, and STITCH database, NSCLC-related genes were obtained from TCGA. The topology analysis strategy, network construct and prognostics analysis were conducted to filter and sort the key genes and underlying molecular mechanisms. In addition, molecular docking analysis provided a new perspective to elucidate the possibility of compounds binding to targets.
Results: The study systematically investigates the potential key components and targets of PN treating NSCLC. The potential target genes mainly involved IL6, EGF, MMP9, PTGS2, JUN, IL1B, FOS, EZH2, CCL2, ICAM1, CDK4, SPP1, CCNB1, AR, PPARG, CDK1 and KDR. The bioinformatics analysis demonstrated that the physiological effects were closely related to synergistic regulation of signal transduction, inflammatory response and oxidation-reduction process. More importantly, the results suggested that PTGS2, JUN and IL6 might be the more important target genes, quercetin and luteolin are probably the more useful compounds, and TNF signaling pathway might play a significant role in PN-mediated anti-cancer effects based on the network and prognostic analysis. Besides, molecular docking analysis results presented binding affinity values of each of the seventeen key genes with their respective compounds.
Conclusions: The present study shows that PN may act on inflammatory response or oxidation process through the TNF signaling pathway, which makes PN a potential treatment strategy for NSCLC. This study offers new insights into PN for future experimental research and provides a scientific basis for more widespread clinical application in the treatment of NSCLC.

Keywords: Plumula nelumbinis, NSCLC, Network pharmacology, Molecular docking

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Introduction

Non-small cell lung cancer (NSCLC) is the main type of cancer for men and women worldwide, and accounts for over 80% of all lung cancer cases [1]. The latest data from the American Cancer Society showed that the incidence and mortality rate of lung cancer patients have decreased from 2014 to 2018; nevertheless, NSCLC still causes the most deaths, far more than any other types of cancer [2]. The alterations in the physiological effect of NSCLC cells and the underlying mechanisms are complex and variable, and the changes of molecular networks may run through the entire process of cancer development, evolution and treatment [3]. Therefore, a better understanding of the underlying molecular mechanisms driving this process and the development of drugs with low side effects are essential to improve the prognosis of patients with NSCLC.

Traditional Chinese medicine has been widely used by Chinese doctors to treat various diseases for many years, and recently it has attracted the attention of western clinicians and researchers. Some traditional Chinese medicines are appealing due to their wide applications and low side effects [4]. Substantial evidence has demonstrated that Chinese herbal medicine ingredients could be used to treat cancer, and it is considered to be a great treasure house for discovering novel therapeutic agents [5-8]. Plumula Nelumbinis (PN), commonly known as Lian-Zi-Xin (lotus seed) in China, is derived from the mature green embryo of Nelumbonaceae plants that mainly grow in southern China and some areas in India [9]. It has been reported that PN can lower blood pressure, decrease the accumulation of body fat, and boost immunity. Therefore, it is often used as a raw material for making health products [9]. In addition, studies have confirmed that certain compounds of PN have anti-tumor effects. *Zhang et al*. reported that isoliensinine, the active compound of PN, exhibited anti-tumor effects in triple-negative breast cancer cells by inducing apoptosis [10]. *Zhou et al*. reported that liensinine could increase the mitophagosomes by blocking autophagy flux, thereby accelerating the death of breast cancer cells [11]. In addition, nuciferin, another compound derived from PN, inhibited the proliferation of neuroblastoma and colorectal cancer cells by affecting the PI3K/AKT pathway [12]. All these findings indicate that PN may have potent pharmacological effects on cancer. However, the physiological effects and molecular mechanisms of PN on NSCLC have not been systematically elucidated.

In recent years, network pharmacology, as an analysis strategy based on disease-drug-target gene construction networks [13], has attracted the attention of researchers. It is believed that network pharmacology has the ability to reveal the interaction between clinical diseases and bioactive medicines [14,15]. In this study, we performed this analysis strategy and presented the results of the interactions of PN in NSCLC at the molecular level.

Materials and Methods

Screening of chemical ingredients

To screen the active compounds of PN, we employed a commonly used pharmacology platform of traditional Chinese medicine, the Traditional Chinese Medicine System Pharmacology Database (TCMSP) [39]. Using two key parameters, Oral bioavailability (OB) and drug-likeness (DL) [40,41], we selected 11 compounds (MOL000006, MOL000098, MOL002419, MOL007206, MOL007213, MOL009155, MOL009156, MOL009157, MOL009160, MOL009167, MOL009172) based on the threshold values of OB exceeding 30% and DL exceeding 0.18 [42]. Next, we selected 7 main components of plumula nelumbinis (MOL000003, MOL000422, MOL002562, MOL009168, MOL009169, MOL009170, MOL009171) through literature search [11,43-46]. Taken together, a total of 18 chemical components of PN were screened out for further study.

Prediction of potential targets

TCMSP database [39], the PubChem database [47] and the STITCH database [48] were used to predict the potential targets of screened chemical components. After that, the targets union were put into Uniprot database [49] to normalize the gene information.

Screening of DEGs in NSCLC

We used mRNA expression data of 1091 NSCLC samples downloaded from the TCGA database (https://www.cancer.gov/tcga). We processed and analyzed mRNA expression data by using edgeR package [50] and GDCRNATools [51]. DEGs of the dataset were selected basing on the cut-off criteria: $|FC| > 2$ and $P < 0.01$. The volcano plot was established using SangerBox.

PPI analysis and network construction

First, the potential targets of PN were mapped to the NSCLC-related DEGs, and the overlapped genes were considered as the candidate targets responsible for PN therapy in NSCLC patients. We used a Venn diagram to intuitively present the number of these overlapping targets. Then these overlapping targets were put into the STRING tool, an online analysis platform that could predict the interaction of proteins we input and generate a PPI network [52]. The Cytoscape3.2.1, another software that could analyze and embellish the original files generated in STRING tools [53], was performed to further processing the compound-target network, compound-NSCLC-overlap network and compound-gene-pathway network. The topological properties analysis was performed in the compound-NSCLC-overlap network by applying the cytoscape

plug-in Network Analyzer (the data was shown in Table S3). The topological properties of every node were assessed by Degree, Betweenness Centrality, Closeness Centrality and selected hub genes for further molecular docking analysis.

Bioinformatic analysis

We performed GO enrichment analysis and KEGG pathway analysis in an online platform, Annotation Visualization and Integrated Discovery (DAVID 6.8 online) [54], to determine the possible biological function of PN against NSCLC. The results of GO enrichment analysis and KEGG pathway analysis were plotted for visualization in this platform: http://www.bioinformatics.com.cn.

Molecular docking

Ligand preparation: We acquired the 2D structures of chemical ingredients by using the PubChem database and the ChemDraw19.0 software. The 2D structure was transferred to 3D chemical structure and make energy minimizing using the Chem3D19.0 software.

Receptor preparation: Firstly, we acquired the crystal structures of hub genes from the RCSB Protein Data Bank [55]. Second, the crystal structures were embellished to eliminate the needless ligand, water molecules and phosphates by PyMol2.3.0, a protein visualization software [56]. The AutoDockTools-1.5.6 [57] was used to add hydrogen and set docking paraments for the receptors. Moreover, grid box of the receptors was set to appropriate to perform the blind docking.

Molecular docking: Autodock Vina, a widely used free reliable data analysis package [58], was performed to calculate the binding affinity of ligand-receptor interaction. And then, we used PyMol2.3.0 software to generate a 3D binding figure and used Discovery Studio3.5 software to generate a 2D binding figure of the docking results.

Kaplan-Meier survival curve analysis

Kaplan-Meier survival curve analysis was performed to assess the correlation between the expression of the 54,000 genes on the survival rates in 21 different cancers using more than 10,000 cancer samples, including 371 liver, 1440 gastric, 3452 lung, 2190 ovarian, and 6234 breast cancer samples. Kaplan-Meier plots (http://kmplot.com/analysis/) were used to analyze the relationship between our 17 potential target genes and overall survival rates in NSCLC based on the hazard ratios (HR) and log-rank P-values [59].

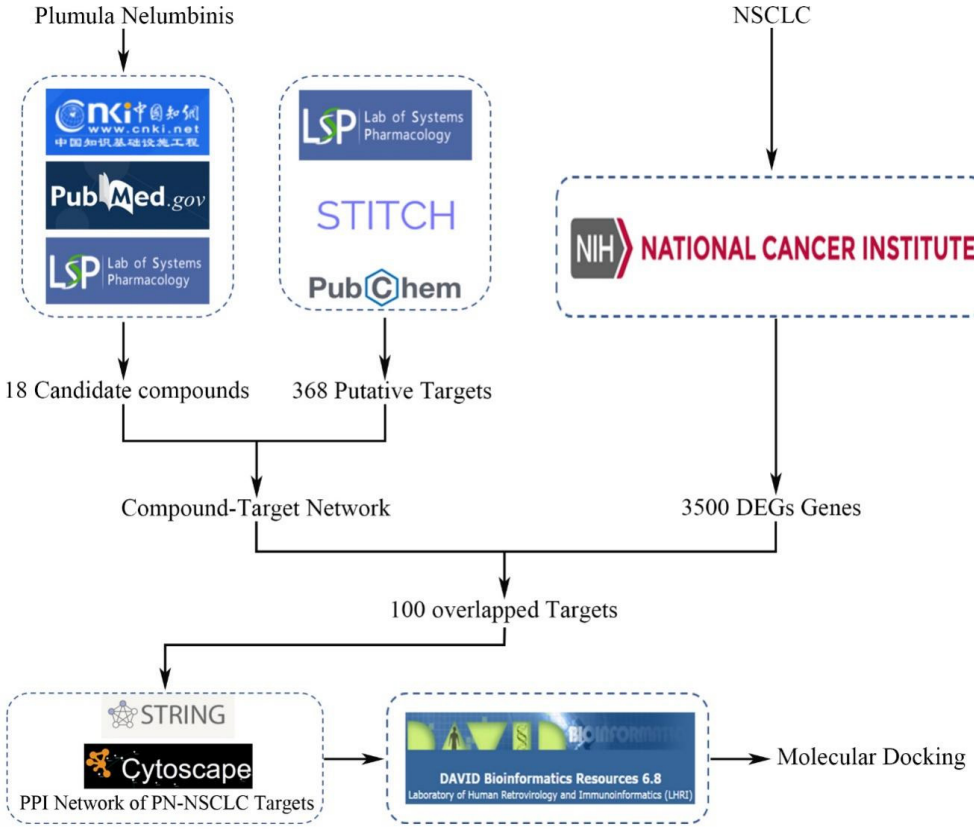
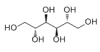
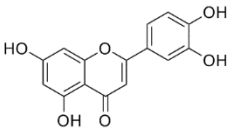
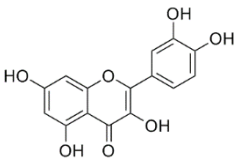
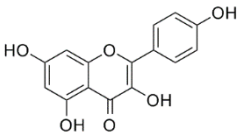
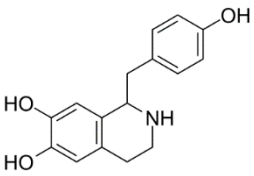
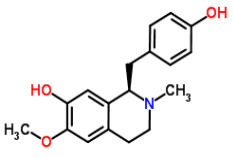
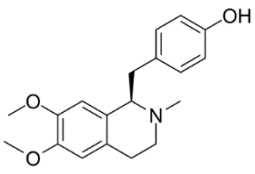
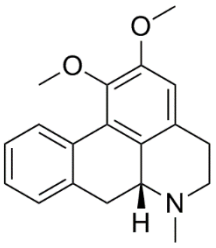
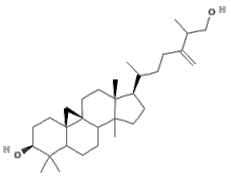
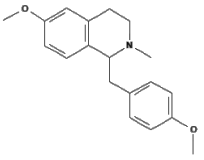
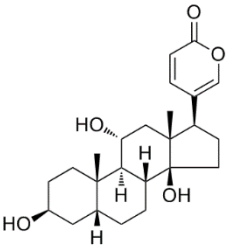
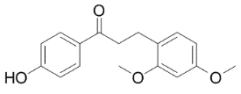


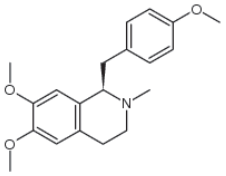
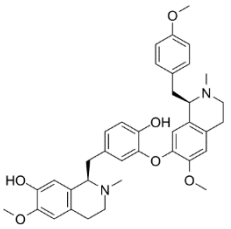
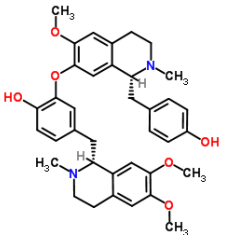
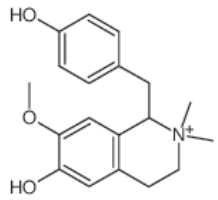
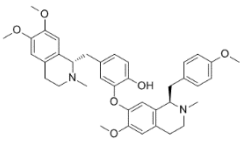
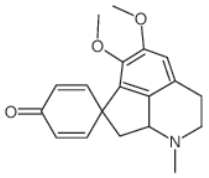
Figure 1

Figure 1. The flow chart showing the process of exploring the potential active components of PN and the underlying molecular mechanisms against NSCLC

Table 1. Chemical information of the compounds of NP.

Number	Mol ID	Compound	Composition	Structure
1	MOL000003	D-mannitol	$C_6H_{14}O_6$	
2	MOL000006	Luteolin	$C_{15}H_{10}O_6$	
3	MOL000098	Quercetin	$C_{15}H_{10}O_7$	
4	MOL000422	Kaempferol	$C_{15}H_{10}O_6$	
5	MOL002419	Higenamine	$C_{16}H_{17}NO_3$	
6	MOL002562	(R)-N-Methylcoclaurine	$C_{18}H_{21}NO_3$	

7	MOL007206	Armepavine	$C_{19}H_{23}NO_3$	
8	MOL007213	Nuciferin	$C_{19}H_{21}NO_2$	
9	MOL009155	14-Methyl-24-methylene-dihydro-mangiferodiol	$C_{33}H_{28}O_2$	
10	MOL009156	4’-methyl-N-methylcoclaurine	$C_{19}H_{23}NO_2$	
11	MOL009157	Gamabufotalin	$C_{24}H_{34}O_5$	
12	MOL009160	Loureirin A	$C_{17}H_{18}O_4$	

13	MOL009167	O-Methylarmepavine	$C_{20}H_{25}NO_3$	
14	MOL009168	Isoliensinine	$C_{37}H_{42}N_2O_6$	
15	MOL009169	Liensinine	$C_{37}H_{42}N_2O_6$	
16	MOL009170	Lotusine	$C_{19}H_{24}NO_3+$	
17	MOL009171	Neferin	$C_{38}H_{44}N_2O_6$	
18	MOL009172	(+)-Pronuciferine	$C_{19}H_{21}NO_3$	

Results

The network of the compounds and target genes

The research protocol was shown in Fig. 1. We selected eleven compounds of PN (MOL000006, MOL000098, MOL002419, MOL007206, MOL007213, MOL009155, MOL009156, MOL009157, MOL009160, MOL009167, MOL009172) from the TCMSP database based on the values of OB exceeding 30% and DL exceeding 0.18. With the application of the HPLC technology, a large number of PN components have been identified over the years. We

selected seven components of PN (MOL000003, MOL000422, MOL002562, MOL009168, MOL009169, MOL009170, MOL009171) through the literature search and added them to the database filtering results. The chemical information for these eighteen compounds of NP was shown in Table 1. We then used the TCMSP, PubChem, and STITCH databases to collect and summarize the potential targets of each compound. The detailed information of a total of 368 targets was described in Table S1. After the construction of the compound-target network and analysis of the 18 active ingredients and 368 targets, the top three components according to the degree values were Quercetin (degree=279), Luteolin (degree=143) and Kaempferol (degree=84).

Screening of DEGs in NSCLC

We processed and analyzed the mRNA expression data of 1091 NSCLC samples from the TCGA database. A total of 3500 DEGs of the NSCLC were selected, the detailed information was shown in Table S2. A total of 1388 up-regulated genes and 2112 down-regulated genes were shown in the volcano plot (Fig. 2B).

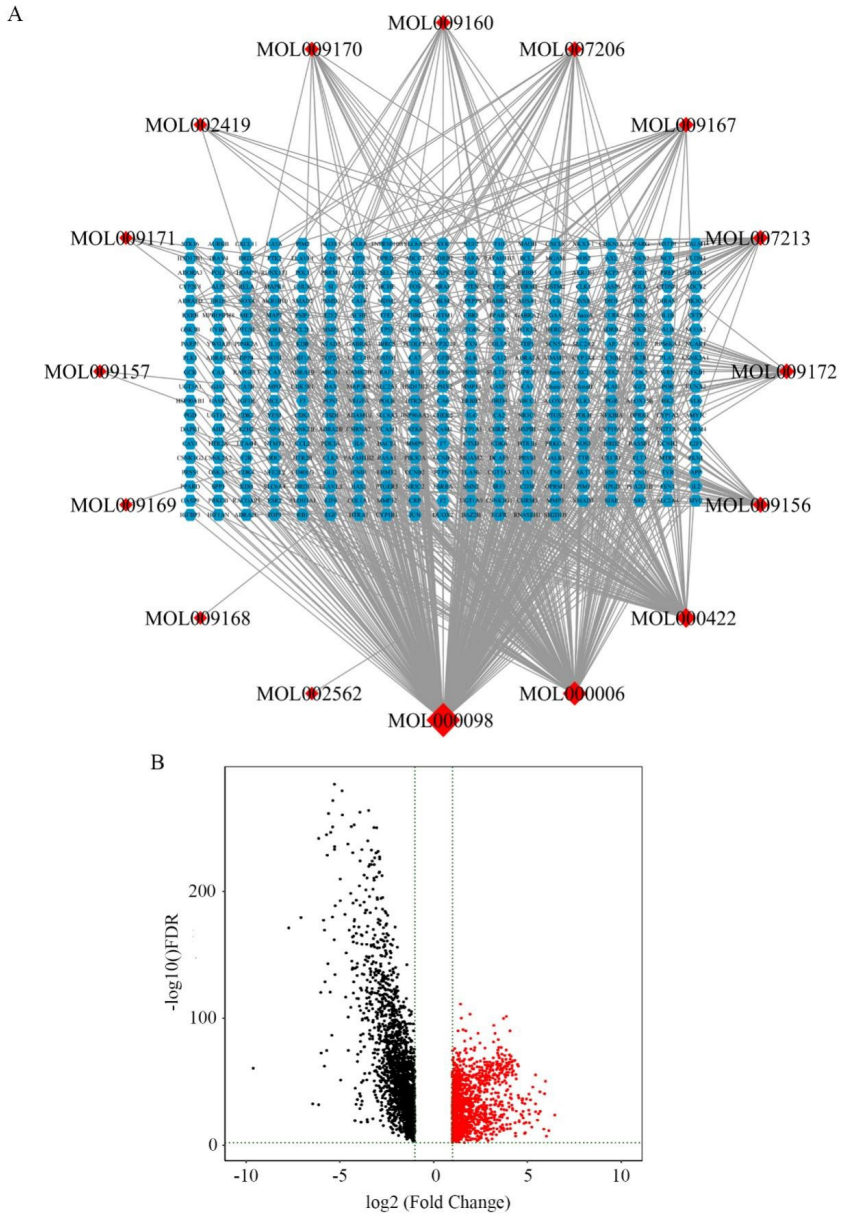


Figure 2

Figure 2. Compound-target network of PN and differentially expressed genes of NSCLC. (A) A network combines 18 compounds with target genes. Red rhombus nodes are compounds of plumula nelumbinis and blue hexagon nodes are target genes. (B) A Volcano plot presents the expression fold-change of mRNA. The red dots are up-regulated genes and the black dots are down-regulated genes.

The PPI network of overlapping molecules

To further identify the molecular targets related to PN treatment of NSCLC and narrow down the number of targets, we combined NSCLC-related target genes with PN-related target genes to intersect the overlapping genes (Fig. 3A). We put these 100 highly correlated target genes into the Strings tool and generated a PPI network to evaluate the interaction between them. Then we used Cytoscape to further analyze their topological feature and arrange these genes according to their degrees (the detailed information was shown in Table S3). As shown in Fig. 3B, we presented these 100 targets in three concentric circles (as the degree value decreases, the area becomes smaller and the color gradually becomes orange). The target genes with a degree value greater than 25 were listed, including IL6, EGF, MMP9, PTGS2, JUN, IL1B, FOS, EZH2, CCL2, ICAM1, CDK4, SPP1, CCNB1, AR, PPARG, CDK1 and KDR. These hub genes were selected for molecular docking analysis.

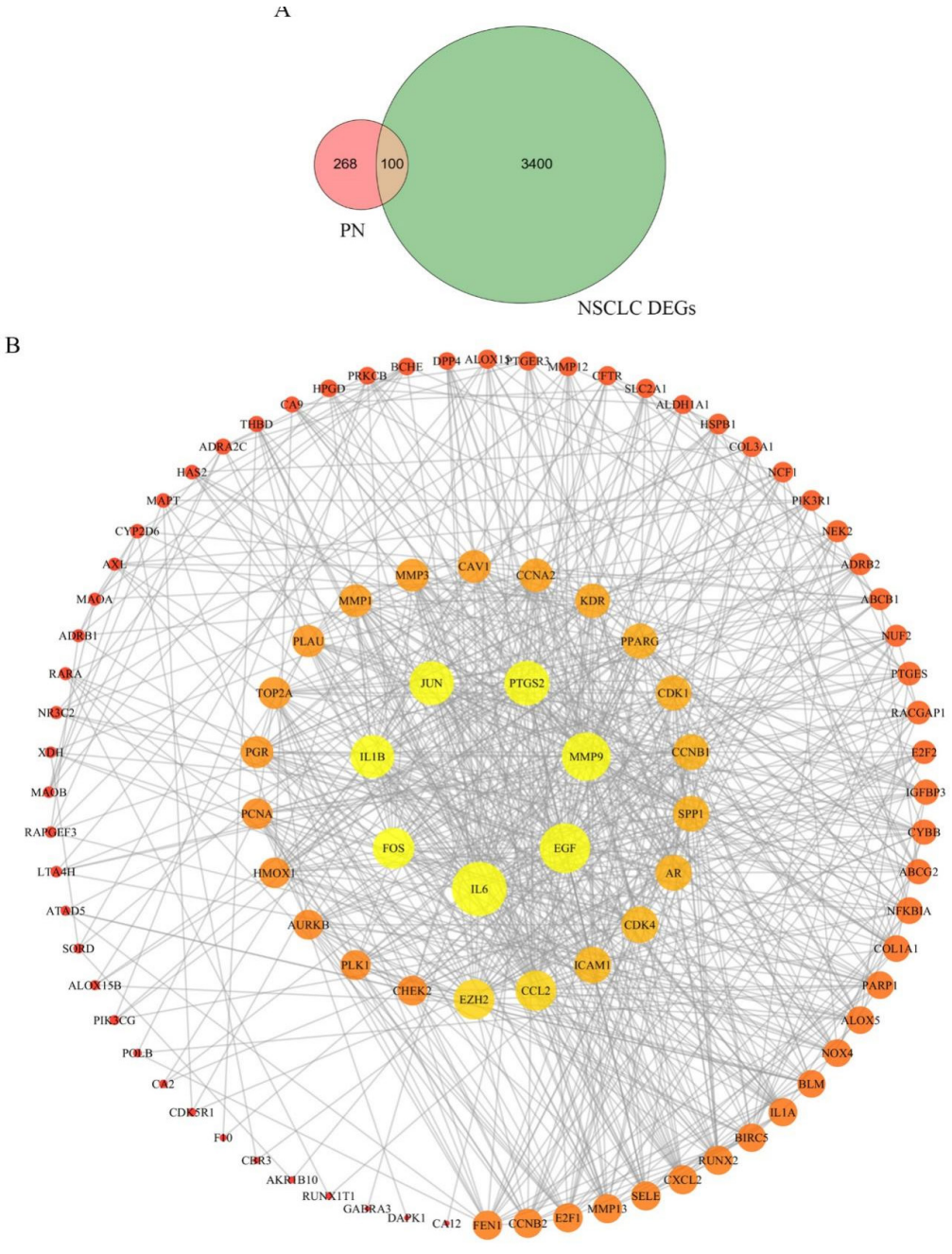


Figure 3

Figure 3. Intersection analysis of compounds of PN and NSCLC-related genes. (A) Venn diagram. (B) A PPI network of the overlapping genes. Arranging the concentric circles according to the degree value. The bigger the value, the bigger the node and more golden the color becomes.

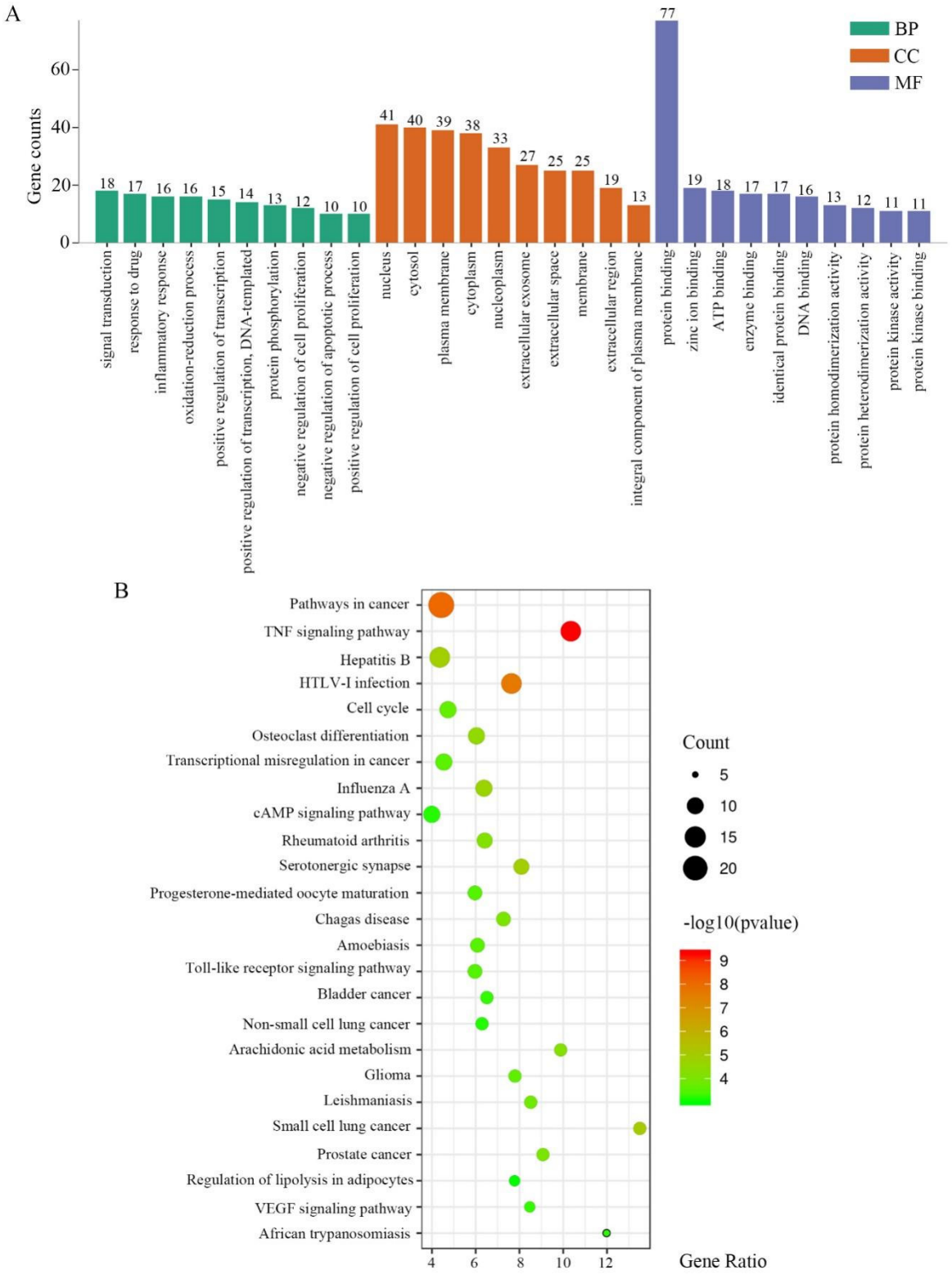


Figure 4

Figure 4. Bioinformatic analysis of overlapping targets. (A) Gene ontology analysis of overlapping targets. The top 10 items of molecular function, cellular component and biological process analysis results. (B) KEGG pathway analysis of overlapping targets. The vertical axis displays the enriched pathways; the horizontal axis displays the enrichment score. MF: Molecular function; CC: Cellular component; BP: Biological process. KEGG Pathway: Kyoto Encyclopedia of Genes and Genome Pathway.

The bioinformatic analysis of 100 hub genes

The Annotation Visualization and Integrated Discovery system has been widely used to analyze the potential biological functions of gene clusters. We next put the overlapped targets in this system to explore the possible mechanism of PN treatment of NSCLC. As shown in Fig. 4A, GO enrichment analysis indicated that these genes were mainly involved in signal transduction, inflammatory response, and oxidation-reduction process. In the cell component module, these genes were mostly enriched in nucleus and cytosol. In the molecular function module, the functions of these genes were mainly related to protein binding. Furthermore, the results of KEGG pathway enrichment analysis displayed 25 pathways according to the P value <0.001 (Table 2, Fig. 4B). The pathways highly related to tumors included the TNF signaling pathway, the transcriptional mis-regulation in the cancer pathway, the cAMP signaling pathway, the Toll-like receptor signaling pathway, the arachidonic acid metabolism pathway, and the VEGF signaling pathway. These data suggested that these pathways could be the underlying mechanisms involved in PN treatment for NSCLC.

The molecular docking analysis of 17 hub genes

Molecular docking is a simulation method for predicting the binding pattern of receptors and ligands. In recent years, it has been mainly used to design and screen drugs by analyzing the properties and interactions of electric field force. Hence, we performed the molecular docking analysis to study the interaction between the target genes and their active compounds in our system. After preparing the files of the receptors and ligands through Autodock tools, Autodock vina tool was performed for docking, and then the docking affinity values were calculated. A total of forty ligand-receptor pairs were delivered into the docking system and results were shown in Table 3. The absolute value of docking affinity was used to reflect the degree of direct binding between ligands and receptors. As shown in Fig. 5,

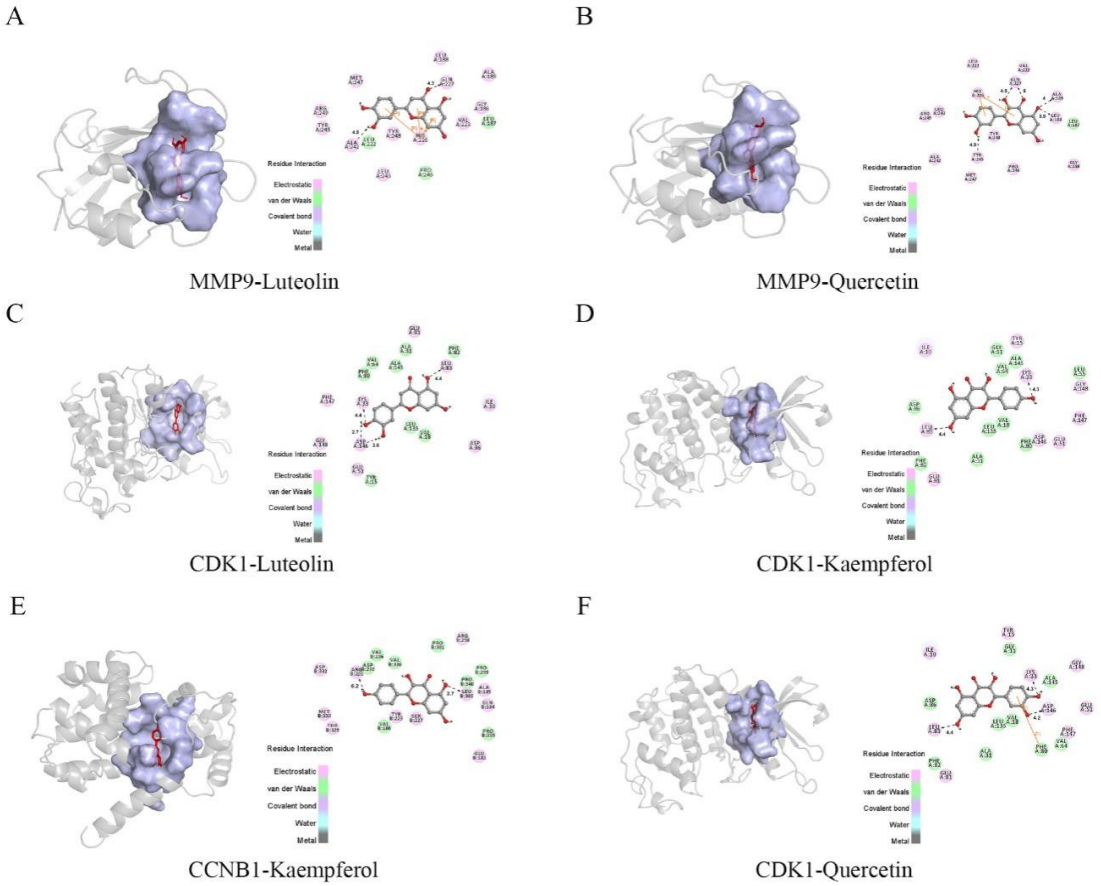


Figure 5

Figure 5. Molecular docking for the representative complexes. (A-F) The 3D view and 2D view of six pairs of ligand-receptor with the best docking affinity were shown. 3D pattern diagram (on the left): The gray lines are the spatial structure of the proteins. The red models in the middle pocket are compounds which binding to the proteins. The amino acid residues around the compounds are shown as blue in color. 2D pattern diagram (on the right): The amino acids residues were bound to the compounds by different connecting methods. The hydrogen bonds were displayed by black dashed lines and the pi-pi stacking interactions were shown by orange lines.

Table 2. The information of top pathways of KEGG analysis.

Number	Pathway Name	Count	Hub Genes	P Value
1	TNF signaling pathway	14	JUN, MMP3, PIK3R1, FOS, PTGS2, SELE, CXCL2, MMP9, PIK3CG, ICAM1, NFKBIA, IL6, IL1B, CCL2	5.04E-10
2	Pathways in cancer	22	JUN, PRKCB, DAPK1, MMP1, EGF, PTGER3, SLC2A1, PIK3R1, FOS, PTGS2, MMP9, PIK3CG, NFKBIA, AR, IL6, CDK4, E2F1, RARA, BIRC5, E2F2, PPARG, RUNX1T1	8.50E-09
3	Hepatitis B	14	JUN, PCNA, PRKCB, PIK3R1, FOS, MMP9, PIK3CG, NFKBIA, CCNA2, IL6, CDK4, E2F1, BIRC5, E2F2	2.20E-08
4	Bladder cancer	7	MMP1, CDK4, DAPK1, EGF, E2F1, E2F2, MMP9	1.01E-05
5	Rheumatoid arthritis	9	IL1A, IL6, JUN, MMP1, IL1B, MMP3, CCL2, FOS, ICAM1	1.22E-05
6	HTLV-I infection	14	JUN, PCNA, SLC2A1, PIK3R1, FOS, PIK3CG, ICAM1, POLB, NFKBIA, IL6, CDK4, CHEK2, E2F1, E2F2	1.37E-05
7	Cell cycle	10	CCNA2, CCNB2, CCNB1, PCNA, CDK4, CHEK2, PLK1, CDK1, E2F1, E2F2	2.15E-05
8	Osteoclast differentiation	10	NFKBIA, IL1A, JUN, NCF1, IL1B, CYBB, PPARG, FOS, PIK3R1, PIK3CG	3.33E-05
9	Non-small cell lung cancer	7	PRKCB, CDK4, EGF, E2F1, E2F2, PIK3R1, PIK3CG	6.30E-05
10	Serotonergic synapse	9	MAOB, MAOA, PRKCB, CYP2D6, ALOX5, ALOX15, PTGS2, ALOX15B, RAPGEF3	6.66E-05
11	Progesterone-mediated oocyte maturation	8	CCNA2, CCNB2, CCNB1, PLK1, CDK1, PGR, PIK3R1, PIK3CG	9.71E-05
12	Arachidonic acid metabolism	7	ALOX5, ALOX15, LTA4H, PTGS2, ALOX15B, PTGES, CBR3	1.03E-04
13	Glioma	7	PRKCB, CDK4, EGF, E2F1, E2F2, PIK3R1, PIK3CG	1.47E-04
14	Transcriptional misregulation in cancer	10	IL6, HPGD, PLAU, IGFBP3, MMP3, RARA, PPARG, MMP9, RUNX2, RUNX1T1	2.19E-04
15	Leishmaniasis	7	NFKBIA, IL1A, JUN, NCF1, IL1B, FOS, PTGS2	2.39E-04
16	Influenza A	10	NFKBIA, IL1A, IL6, JUN, PRKCB, IL1B, CCL2, PIK3R1, PIK3CG, ICAM1	2.97E-04
17	Chagas disease (American trypanosomiasis)	8	NFKBIA, IL6, JUN, IL1B, CCL2, FOS, PIK3R1, PIK3CG	2.98E-04
18	Amoebiasis	8	COL1A1, COL3A1, IL6, PRKCB, IL1B, HSPB1, PIK3R1, PIK3CG	3.35E-04
19	Toll-like receptor signaling pathway	8	NFKBIA, IL6, JUN, IL1B, SPP1, FOS, PIK3R1, PIK3CG	3.35E-04
20	Regulation of lipolysis in adipocytes	6	PTGER3, ADRB1, ADRB2, PIK3R1, PTGS2, PIK3CG	6.29E-04
21	Small cell lung cancer	7	NFKBIA, CDK4, E2F1, E2F2, PIK3R1, PTGS2, PIK3CG	6.36E-04
22	African trypanosomiasis	5	IL6, PRKCB, IL1B, SELE, ICAM1	7.07E-04
23	cAMP signaling pathway	10	NFKBIA, JUN, PTGER3, ADRB1, FOS, ADRB2, PIK3R1, CFTR, RAPGEF3, PIK3CG	7.65E-04
24	Prostate cancer	7	NFKBIA, AR, EGF, E2F1, E2F2, PIK3R1, PIK3CG	7.65E-04
25	VEGF signaling pathway	6	PRKCB, KDR, HSPB1, PIK3R1, PTGS2, PIK3CG	9.33E-04

Table 3. Molecular docking of 17 hub genes and compounds of NP.

Number	Hub Gene	PDB ID	Compound	Docking affinity (kcal/mol)
1	IL6	1IL6	Luteolin	-7.4
			Quercetin	-7.4
2	EGF	1P9J	Quercetin	-5.6
3	MMP9	6ESM	Luteolin	-10.8
			Quercetin	-10.7
4	PTGS2	5F19	Luteolin	-9.6
			Quercetin	-9.6
			Armepavine	-8.5
			Nuciferin	-9.6
			4’-methyl-N-methylcoclaurine	-8.7
			Loureirin A	-8.3
			O-Methylarmepavine	-8.7
			Lotusine	-8.5
			(+)-Pronuciferine	-8.8
5	JUN	1JUN	Luteolin	-5.6
			Quercetin	-5.5
6	IL1B	5BVP	Quercetin	-7
7	FOS	1A02	Luteolin	-5.6
			Quercetin	-5.2
8	EZH2	4MI0	Quercetin	-7.7
9	CCL2	1DON	Quercetin	-7.2
10	ICAM1	1IAM	Luteolin	-7
			Quercetin	-6.8
11	CDK4	2W9F	Luteolin	-8
12	SPP1	6UH5	Quercetin	-6.8
13	CCNB1	6GU2	Luteolin	-9.3
			Quercetin	-9.6
			Kaempferol	-9.9
14	AR	1T65	Luteolin	-8.9
			Quercetin	-9
			Kaempferol	-8.5
			Nuciferin	-9
			4’-methyl-N-methylcoclaurine	-8.2
15	PPARG	2QMV	Luteolin	-8.3
			Quercetin	-8.4
			Kaempferol	-8.2
16	CDK1	4Y72	Luteolin	-10.5
			Quercetin	-9.9
			Kaempferol	-10
17	KDR	1YWN	Quercetin	-8.1

we used Pymol software and Discover Studio software to generate the 2D and 3D pattern diagrams of the top six receptor-ligand binding complexes, including MMP9-Luteolin docking (-10.8 kcal/mol), MMP9-Quercetin docking (-10.7 kcal/mol), CDK1-Luteolin docking (-10.5 kcal/mol), CDK1-Kaempferol docking (-10 kcal/mol), CCNB1-Kaempferol docking (-9.9 kcal/mol), CDK1-Quercetin docking (-9.9 kcal/mol). In order to better present our docking results, we took one complex of our results as an example for showing more details. As shown in Fig.5B (left panel), the 3D pattern diagram indicates that the red-marked molecule Quercetin binds to the cyan-marked MMP9 in a pocket-like region through interacting with amino acid residues. On the right, the 2D pattern diagram indicates that Quercetin and MMP9 are boned by five hydrogen between four amino acid residues, including TYR245, LEU 188, ALA 189 and GLN 227. Moreover, HIS 226 amino acid residues interact with the two benzene rings of Quercetin by forming two pi-pi stacks. The other essential amino acid residues of MMP9 bonded with Quercetin via electrostatic forces or van der Waals forces include LEU 222, VAL223, LEU 187, GLY 186, PRO 246, TYR 248, MET 247, ALA 242, ARG 249, and LEU 243. Taken together, the ability of binding affinity depends on the diversity of interaction forms, and hydrogen bonding and electrostatic force may play the most important role.

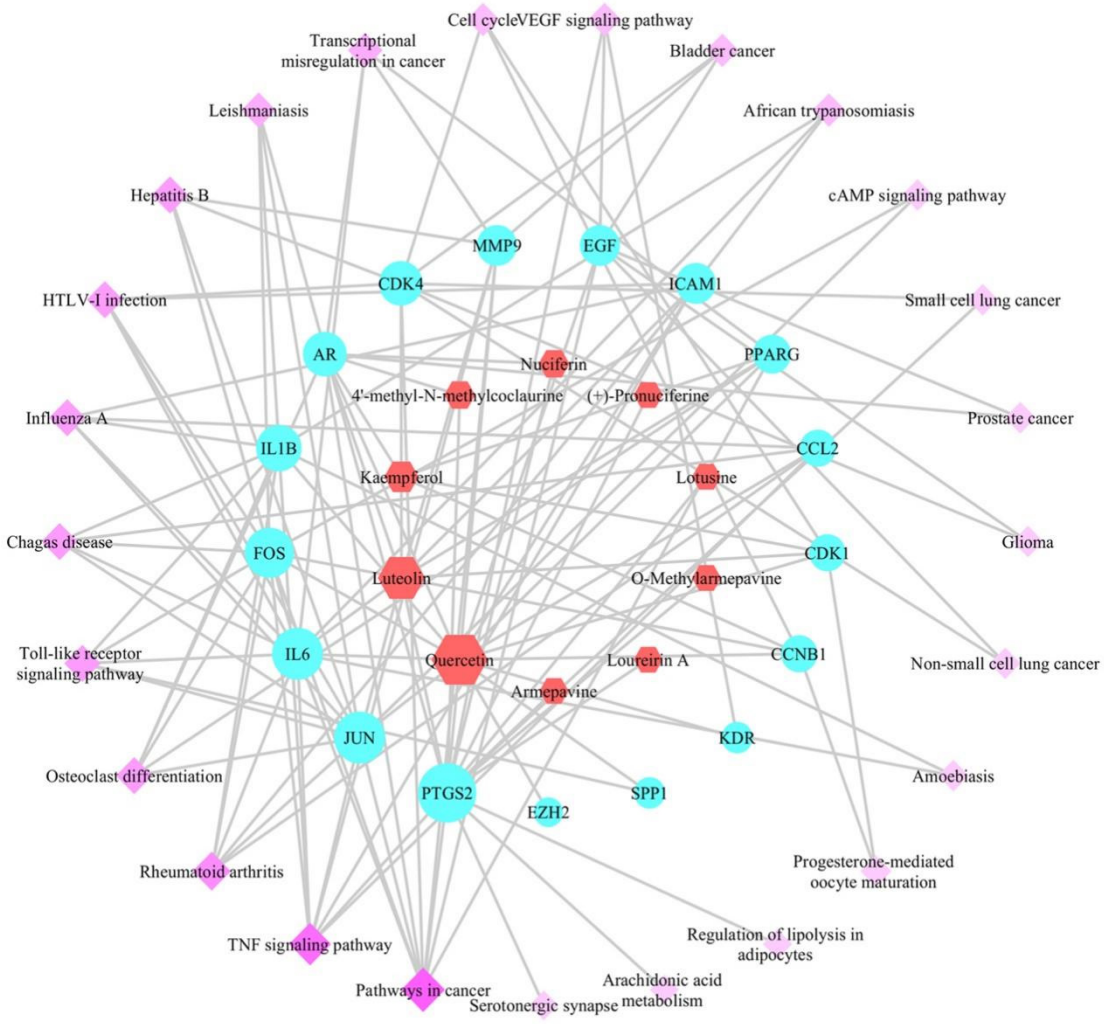


Figure 6

Figure 6. The network consists of hub compounds, target genes, and pathways. Selected compounds-hub genes-pathways network. The compounds were displayed by hexagon nodes. The hub genes were displayed by circular nodes. The hub pathways were displayed by rhombus nodes. The sizes of node are correlated with the degree values.

The compound-gene-pathway network and prognostic analysis

The three elements identified above (pathways, compounds, and target genes) were put together to construct a compound-target-pathway network (Fig. 6). We could use respective average degree to define the major nodes in order to identify the most significant nodes. We present them according to the degree scores, including Quercetin (degree=16), Luteolin (degree=11), Kaempferol (degree=4), PTGS2 (degree=17), JUN (degree=13), IL6 (degree=13), FOS (degree=12), IL1B (degree=10), AR (degree=9), and CDK4 (degree=9). Besides, we analyzed the prognostic value of 17 potential target genes expression using the Kaplan-Meier plotter database (Figure S1A-S1Q). If we set P value is 0.005 as a cutoff. High IL6 (OS: HR=1.32, 95% CI=1.16-1.49, P=2e-05), EZH2 (OS: HR=1.31, 95% CI=1.15-1.48, P=3.8e-05), CDK4 (OS: HR=1.51, 95% CI=1.33-1.71, P=2.1e-10), SPP1 (OS: HR=1.34, 95% CI=1.18-1.52, P=5.2e-06), CCNB1 (OS: HR=1.62, 95% CI=1.37-1.91, P=8.7e-09), CDK1 (OS: HR=1.4, 95% CI=1.23-1.59, P=2.3e-07) expression was associated with poorer prognosis in NSCLC. Low PTGS2 (OS: HR=0.81, 95% CI=0.71-0.92, P=0.001), JUN (OS: HR=0.79, 95% CI=0.7-0.9, P=0.00023), KDR (OS: HR=0.69, 95% CI=0.61-0.78, P=6.3e-09) expression was associated with poorer prognosis in NSCLC. However, EGF (OS: HR=0.88, 95% CI=0.78-1, P=0.048), MMP9 (OS: HR=1.14, 95% CI=1-1.29, P=0.046), ILB1B (OS: HR=1, 95% CI=0.88-1.14, P=0.97), FOS (OS: HR=0.9, 95% CI=0.79-1.02, P=088), CCl2 (OS: HR=1.13, 95% CI=1-1.28, P=0.06), ICAM1 (OS: HR=0.84, 95% CI=0.74-0.95, P=0.0054), AR (OS: HR=1.13, 95% CI=1-1.28, P=0.056), PPARG (OS: HR=0.94, 95% CI=0.83-1.07, P=0.37) expression was not associated with overall survival in NSCLC. Taken together, these results demonstrate that PTGS2, JUN and IL6 might be the more important target genes, quercetin and luteolin are probably the more useful compounds, TNF signaling pathway might play an important role involved in this system based on network and prognostic analysis.

Discussion

Discovering novel agents and therapeutic targets is essential for the development of therapies for NSCLC patients. Active compounds from traditional Chinese medicine with low side effects can be used to treat a variety of cancers [16]. In the current study, we performed a network pharmacology analysis strategy to explore the potential compounds and targets of plumula nelumbinis in the treatment of NSCLC. After the screening of three traditional Chinese medicine databases and a series of literatures review, 18 components of plumula nelumbinis and 368 potential targets were identified. At the same time, 3500 differentially expressed genes from TCGA database were defined as the key nodes in the process for regulating NSCLC. Furthermore, the 100 overlapping targets were subjected to relevant biological annotation analysis and several PPI networks were constructed. The results of GO enrichment analysis indicated that the targets of plumula nelumbinis were highly related to the regulation of signal transduction, inflammatory response, oxidation-reduction process, protein phosphorylation, protein binding and ATP binding.

In our network analysis, we mainly focused on the top three compounds, including quercetin, luteolin, and kaempferol. Dong et al. demonstrated that quercetin could inhibit the proliferation and metastasis of NSCLC by blocking Src/Fn14/ NF-κB pathway *in vivo* and *vitro* [17]. Recently, a study reported that luteolin enhanced TRAIL sensitivity by increasing DR5 expression and Drp1-mediated mitochondrial fission in NSCLC [18]. Kaempferol could inhibit Nrf2 signaling pathway through decreasing the mRNA expression level of Nrf2, and exhibits a significantly anti-tumor effect in NSCLC cells [19]. Taken together, these active components could be involved in anti-cancer effects of plumula nelumbinis against NSCLC.

Based on the above analysis, we provided a macro perspective on how plumula nelumbinis could achieve therapeutic effects on NSCLC. Interestingly, the KEGG pathway analysis showed that pathways in cancer were the most prominent, which was consistent with the subject of this research. More importantly, we found that TNF signaling pathway may also play an important role in our analysis system. The TNF superfamily contains a large number of cytokines [20], which not only play a conclusive role in hematopoietic function, anti-bacterial infection, immune monitoring and tumor degeneration, but also its maladjustment can lead to a variety of cancers [21]. A variety of inflammatory factors often interact with tumors during their growth to form a special tumor microenvironment [22]. In particular, numerous studies have shown that the tumor-infiltrating immune cells play a leading role in the tumor microenvironment [23]. Considering the multiple studies reported that plumula nelumbinis has anti-inflammatory properties [24-27], we believe that the inflammatory response occurred in cancer cells could be affected with active components of plumula nelumbinis by disturbing the TNF signaling pathway. Proper regulation of TNF could help promote anti-tumor immune responses and improve the efficacy of existing anti-cancer therapies, especially immunotherapies [28]. Therefore, we also audaciously predicted that certain components of plumula nelumbinis may enhance or synergize the immunotherapy of NSCLC, which objectively needs more experimental evidence to support.

Remarkably, three potential hub genes identified based on network and prognostics analysis were all present in TNF pathway, including PTGS2, JUN, IL6. PTGS2 is a highly inducible gene, activated by cytokines, growth factors, oncogenes and chemical carcinogens [29]. Functional studies demonstrated that PTGS2 plays a role in

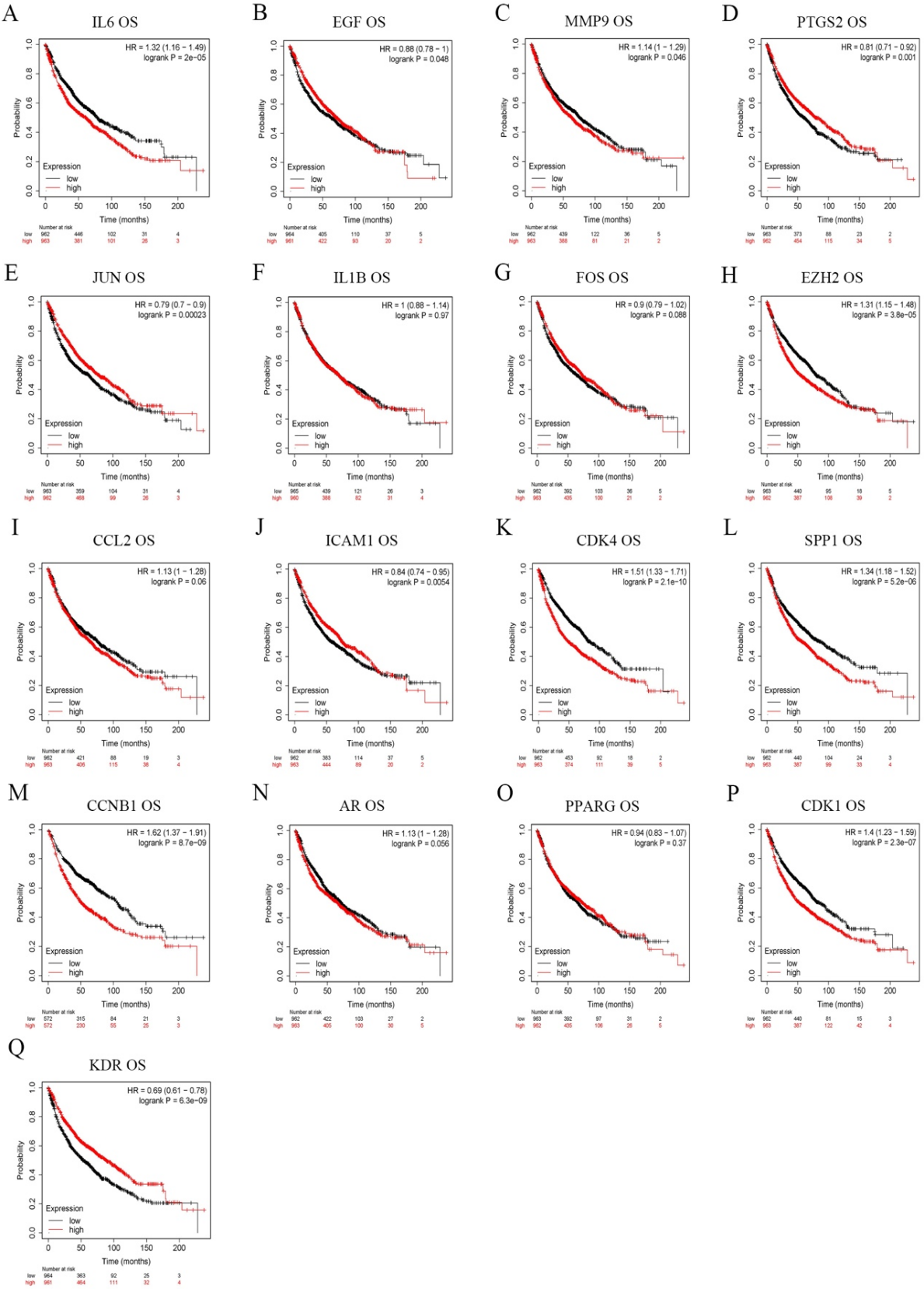


Figure S1

Figure S1. Kaplan-Meier survival curve analyzing the high and low expression of these potential target genes using the Kaplan-Meier plotter database. (A-Q) OS, overall survival.

carcinogenesis and is overexpressed in many human malignancies especially in lung adenocarcinoma [30]. Guo et al. found a potentially synergistic association of PTGS2 polymorphisms with the underlying cause of lung cancer in northeastern Chinese [31]. JUN is a transcription factor that recognizes and binds to the AP-1 consensus motif [32]. Together with FOS family, JUN plays a role in activation-induced cell death of T cells and c-Fos/c-Jun complex could deregulation in NSCLC based on their interactions with other genes [33]. IL6 is a efficient inducer of the acute phase response in immunity. Rapid production of IL6 contributes to host defense but excessive IL6 synthesis is involved in disease pathology including cancer. Study has proved that high IL-6 expression level is associated with worse prognosis in patients with NSCLC [34]. It has also been reported that IL6 production was involved in gefitinib resistance in EGFR-mutant lung cancer cells [35]. In addition to these three TNF-related genes, CDK4, a member of the cyclin-dependent kinase family, has also attracted our attention. The protein encoded by CDK4 is closely related to the regulation of G1 cell cycle progression. *Marval et al*. found that abnormal expression and activity of CDK4 has a strong oncogene function [36]. Many studies have shown that CDK4 is abnormally high-expressed in tumors including lung cancer [37], and inhibiting CDK4 expression can restore the function of tumor suppressor gene p21, thereby achieving the goal of eliminating the malignant phenotype of tumor cells [38]. Therefore, it is reasonable to speculate that compounds of plumula nelumbinis may exert an anti-tumor effect by targeting these genes.

This study has some defects that need to be acknowledged. First, the data used in this study were obtained from public databases, and the timeliness of the databases determine that the results have certain limitations and requires further expansion in the future. Second, the binding and interaction of molecules with target genes does not necessarily reflect what happens in real cancer cells. In addition, further experiments validating the prediction results are necessary

Conclusion

The novel network pharmacology approach implemented in this study has identified several potential compounds and action targets of plumula nelumbinis in the treatment of NSCLC. First, through the screening of traditional Chinese medicine databases and literature review, 18 key components of plumula nelumbinis and 368 potential targets were identified. Moreover, 3500 differentially expressed genes were defined as the key nodes regulating the development of NSCLC. Further, related biological annotation analysis was performed on 100 overlapping targets and several PPI networks were constructed. The analysis results indicated that the molecular targets of plumula nelumbinis were highly related to the regulation of signal transduction, inflammatory response, oxidation-reduction process, protein phosphorylation, protein binding and ATP binding. More importantly, the results suggested that PTGS2, JUN and IL6 might be the more important target genes, quercetin and luteolin are probably the more useful compounds, and TNF signaling pathway might play an important role in PN-mediated anti-cancer effects based on the network and prognostic analysis. Therefore, this study provides new insights into the potential anti-cancer effects of plumula nelumbinis on NSCLC.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Minghui Chang: Methodology, Writing-Original draft preparation. Siyu Chen: Validation, Data curation, Revision. Changhao Li: Data curation, Revision. Yuhan Zhang: Data curation, Revision. Hong Zhao: Supervision. Conceptualization, Funding acquisition and Writing-Reviewing.

Availability of data and materials

Please contact author for data requests.

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