Exploring the Mechanism of Periplogenin in the Treatment of Lung Cancer Based on Network Pharmacology and Molecular Biology

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Lung cancer (the 5-year survival rate is only about 16%) has a low survival rate, and more-effective drugs are urgently needed. Our team discovered that cortex Periplocae Radicis has obvious toxic effects on various cancer cells, including lung cancer cells. However, the mechanism is not clear. Therefore, we used the PubChem database to obtain periplogenin as the target of therapeutic drugs and the TCGA database to obtain differential genes of lung cancer. The results showed that MMP9, PPARG, BMP2, and TGFB2 were the core proteins of periplogenin acting on lung adenocarcinoma (LUAD), and MMP9, angiotensin-converting enzyme (ACE), BMP2, PPARG, MMP13, MMP3, and TGFB2 were the core proteins of periplogenin acting on lung squamous cell carcinoma (LUCS). Through gene ontology (GO) enrichment analysis, it was found that periplogenin mainly acted on LUAD via fatty acid binding, metallopeptidase activity, and monocarboxylic acid binding, and mainly acted on lung squamous carcinoma (LUSC) via endopeptidase activity, metallopeptidase activity, and serine-type peptidase activity. Kyoto Encyclopedia of Genes, and Genomes (KEGG) analysis revealed that the IL-17 signaling pathway, fluid shear stress, atherosclerosis, hepatocellular carcinoma, and so on, were the main signaling pathways of periplogenin acting on LUSC, whereas glycolysis/gluconeogenesis, and the peroxisome proliferator-activated receptor (PPAR) signaling pathway were major signaling pathways of periplogenin acting on LUAD. This shows that treatment of lung cancer can be achieved through multi-targeted, and multi-channel periplogenin activity.

Key words: Periplogenin of Cortex Periplocae; Lung Cancer; Network Pharmacology; Mechanism of Action
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INTRODUCTION

Lung cancer a common cancer throughout the world, and has the highest mortality rate [1]. Lung cancer is broadly classified into two categories: non-small cell lung cancer (NSCLC), which represent 80%–85% of lung cancers; and small cell lung cancer, which comprises the other 15%–20%. At present, it is believed that lung cancer develops due to the combined action of external, and internal influences [2]. Genetic susceptibility, environmental factors, microbial changes, and/or chronic inflammation all increase the risk of cancer [3]. In terms of genetic genes, Kligerman et al. [4] found that the mutation of glutathione transferase M1, high expression of the CYP1A1 gene, mutation of p53, and mutation of the gastrin polypeptide receptor gene increase

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women's genetic, and molecular susceptibility to smoking carcinogenesis compared to that of men, while family history, decreased DNA-repair ability, and the epidermal growth factor receptor (EGFR) mutation are genetic factors that are unrelated to tobacco use. Recently, new lung cancer susceptibility genes were found in genome-wide association studies, including chromosome 6p21, 5p15.33, and 15q24-25.1. It is also important to study the molecular mechanism, and signal transduction pathway of lung cancer to discover valuable biomarkers, and molecular targeted therapy. Previous studies have shown that heat shock protein 90 (Hsp90) expression, which is high in cancer cells, and in the serum of lung cancer patients, is closely related to lung cancer occurrence, and prognosis [5]. In addition, a series of Hsp90 inhibitors have been studied as effective molecular targeted therapy strategies against lung cancer. There are other mechanisms of autophagy, proliferation, apoptosis, and metastasis of lung cancer.

Clinical studies show that [6] the five-year survival rate of stage IIIB, and IV lung cancer patients is 5%–20%, while that of stage I lung cancer patients is as high as 60%–90%. Furthermore, the cure rate of patients with carcinoma *in situ* is nearly 100%. NSCLC has the characteristics of rapid progress, hidden symptoms, and high recurrence rate. Patients have often progressed to the middle or late stage of the disease when they are diagnosed, which greatly hinders the therapeutic effect, and prognosis of lung cancer patients. Therefore, the development of drugs with improved efficacy in treating lung cancer is urgently needed.

At present, the discovery of new antitumor drugs with strong activity, and few side effects it is a research hotspot, and Chinese herbal medicine has attracted attention for its therapeutic derivatives. In our previous study, we screened the monomers of cortex Periplocae, and found that Periplocae had inhibitory effects on various cancer cells, including lung cancer. Cortex Periplocae Radicis has the pharmacological effects of anti-inflammation, rheumatism, edema, diuresis, cardiotonic, pressor, and anthelmintic. As research elucidates the effective components of traditional Chinese medicine, it was found that the extract of cortex Periplocae has an antagonistic effect on catecholamine [7], and can induce apoptosis in gastric cancer cells [8]. Further study on the extract of cortex Periplocae revealed that periplogenin might be a key antitumor compound [9]. Periplogenin is a cardiac glycoside extracted from cortex Periplocae and possesses a structure that is similar to the structure of bufalin, which has been found to induce apoptosis in various tumor cells [10]. However, the effect of periplogenin on lung cancer has not been reported so far. Therefore, we tried to elucidate periplogenin in lung cancer from the perspective of network pharmacology, so as to provide a reference for the exploration of new drugs for lung cancer treatment.

MATERIALS AND METHODS

Screening of Corresponding Targets for Periplogenin

The chemical structure of periplogenin was obtained through the PubChem database (https://pubchem.ncbi.nlm.nih.gov). According to the structure of periplogenin, the data were collected through the PharmMapper database (http://www.lilab-ecust.cn/pharmmapper/), and potential targets corresponding to periplogenin were obtained and stored in the UniProt database (https://www.uniprot.org/). The UniProt target was corrected to the gene name (Gene Symbol).

Screening of Differentially Expressed Genes in Lung Cancer

UCSC Xena (http://xena.ucsc.edu/) TCGA downloads lung cancer data. Objective to screen differentially expressed genes in lung cancer using R version 3.6.0 "limma" package with adj.p.val ≤ 0.05 and |logFC| ≥ 1.

Construction of a Protein Interaction (PPI) Network

Using the merge function of Cytoscape software (version 3.7.2), the intersection of the target of periplogenin and lung cancer was obtained, and the intersection target was transferred to the online string database (https://string-db.org/cgi/input.pl). A PPI core network was constructed, and the core targets of periplogenin on lung cancer were screened by R software.

Bioinformatics Analysis

R software (version 4.0.2), and its software package were used to conduct the enrichment of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of periplogenin acting on lung cancer targets. A false discovery rate (FDR) < 0.05 was used as the threshold for screening significantly enriched items, and R language was used to visually display the top items.

RESULTS AND DISCUSSION

Drug Target Protein

The English name of periplogenin is periplogenin, and its 3D structure SDF file was uploaded. Based on the database construction method, 197 drug targets were obtained (the first 50 are shown in Table 1).

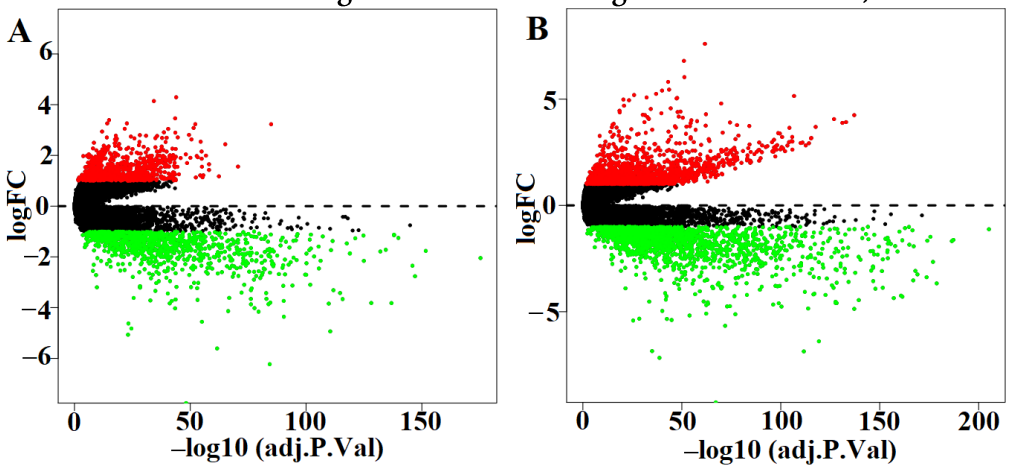
Table 1.
Corresponding targets of periplogenin

Num	TargetSymbol	Fit	NormFit	Num	TargetSymbol	Fit	NormFit
1	THRB	2.978	0.9927	26	CASP7	2.415	0.8051
2	AKR1C2	2.978	0.9925	27	DDX6	2.402	0.8005
3	AR	2.976	0.992	28	SULT2A1	3.989	0.7977
4	CA2	2.964	0.9881	29	HSD17B1	3.187	0.7969
5	STS	2.956	0.9854	30	CCNA2	2.385	0.795
6	CYP19A1	2.919	0.9729	31	PNP	2.383	0.7944
7	ALB	2.912	0.9706	32	NR3C2	3.947	0.7893
8	HSD17B11	2.9	0.9668	33	MAPK1	2.337	0.7791
9	FAP	2.879	0.9595	34	MAPK14	2.323	0.7744
10	APOA2	2.877	0.9589	35	EPHB4	3.079	0.7698
11	CES1	2.842	0.9473	36	SHBG	3.067	0.7668
12	AKR1C3	2.788	0.9292	37	CHEK1	2.26	0.7535
13	MAOB	2.763	0.921	38	CA1	2.257	0.7522
14	KIF11	2.757	0.9191	39	GC	2.996	0.749
15	TTR	2.749	0.9165	40	NOS3	2.96	0.74
16	BMP2	2.748	0.9159	41	ESR1	2.958	0.7396
17	PIM1	2.735	0.9118	42	SOD2	3.684	0.7368
18	EGFR	2.707	0.9024	43	ESR2	2.202	0.7339
19	MAPKAPK2	2.689	0.8964	44	GSTP1	2.198	0.7326
20	TREM1	2.675	0.8917	45	SRC	2.927	0.7318
21	CDK2	2.667	0.8892	46	HSP90AA1	2.193	0.7311
22	NR1H2	2.621	0.8738	47	PPARG	2.193	0.7309
23	PGR	2.592	0.864	48	PDE4D	2.918	0.7294
24	BACE1	2.451	0.8171	49	PPIA	2.187	0.7291
25	CASP3	2.435	0.8118	50	HSPA8	2.859	0.7147

Disease Differential Gene

From UCSC Xena (http://xena.ucsc.edu/), TCGA data of lung cancer were obtained, including lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC). Using adj.p.val ≤ 0.05 and |logFC| ≥ 1 as the threshold, differentially expressed genes in lung cancer were screened (Fig. 1).

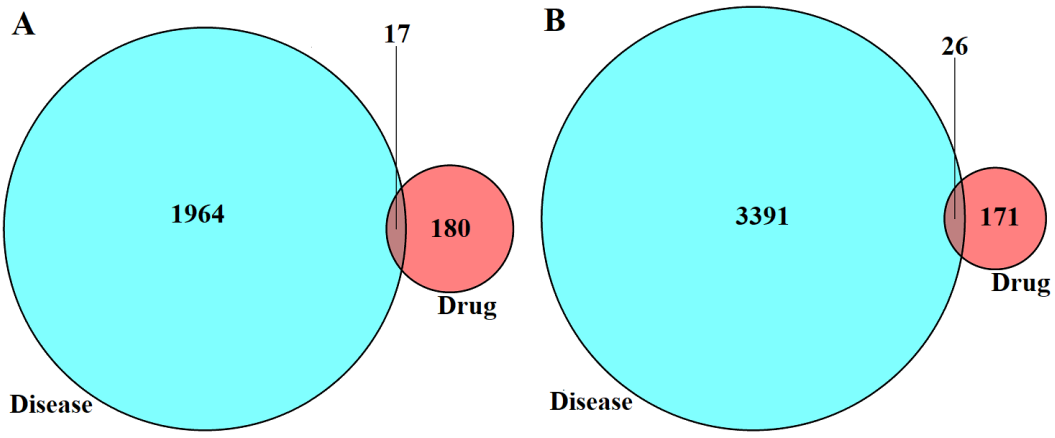
Fig. 1.
Lung cancer differential genes in the TCGA database. A: Differential gene of LUAD. B: Differential gene of LUSC. (The red dots are increased genes, and green dots are decreased genes, besides, black dots are the genes which are no significant difference).



Gene Intersection

By crossing the target genes of periplogenin and lung cancer differential genes, 17 intersection genes of LUAD and periplogenin, and 26 intersection genes of LUSC and periplogenin were obtained (Fig. 2).

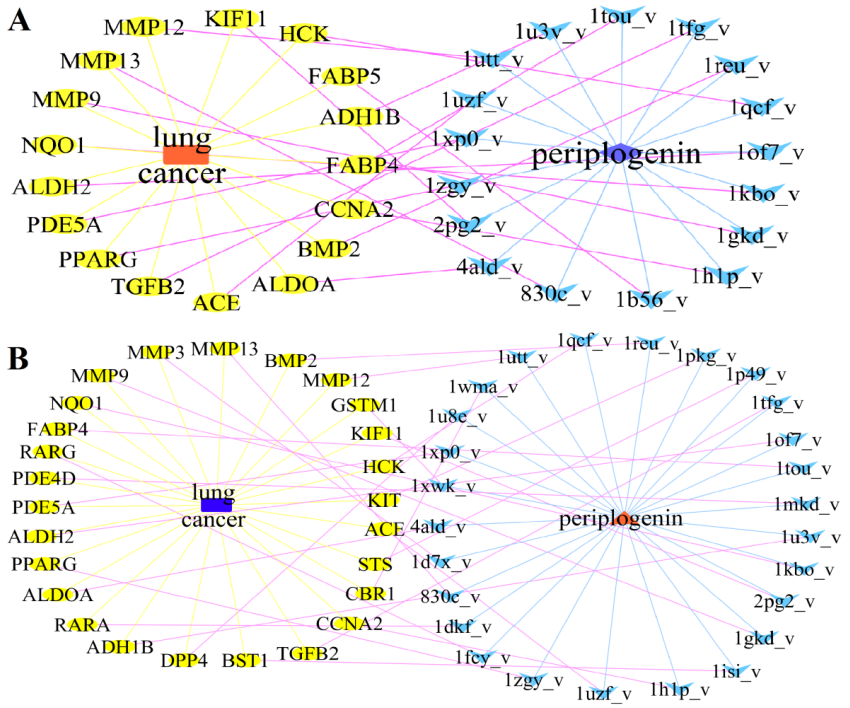
Fig. 2.
Intersection of periplogenin target and lung cancer target. A: Intersection gene of periplogenin and LUAD. B: Intersection gene of periplogenin and LUSC.



Traditional Chinese Medicine Regulatory Network

By using Cytoscape software, we can obtain the regulatory network of traditional Chinese medicine (Fig. 3).

Fig. 3.
"Drug-target-gene-disease" network regulation diagram. A: Regulatory network diagram of periplogenin and LUAD. B: Regulatory network diagram of periplogenin and LUSC.



Protein Interaction Networks and Core Targets

The targets of periplogenin, LUAD and LUSC were input into the string database, And the protein interaction network was obtained (Fig. 4). Each node in the graph represents a protein. The number connecting each node in the protein interaction network was obtained by using R software. It was found that MMP9, PPARG, BMP2, and TGFB2 were the core proteins of periplogenin acting on LUAD (node connection ≥ 4), whereas MMP9, angiotensin-converting enzyme (ACE), BMP2, PPARG, MMP13, MMP3, and TGFB2 were the core proteins of periplogenin acting on LUAD (node connection ≥ 4) (Fig. 5).

Fig. 4.
PPI network. A: Protein interaction network of periplogenin and LUAD. B: Protein interaction network of periplogenin and LUSC.

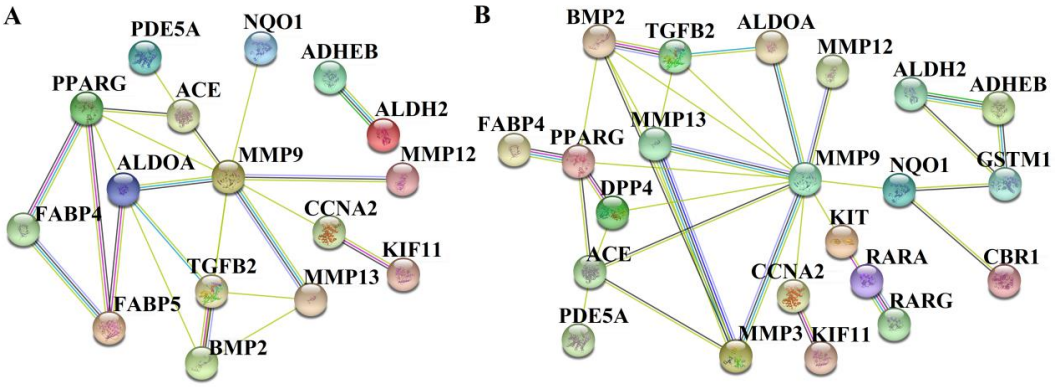
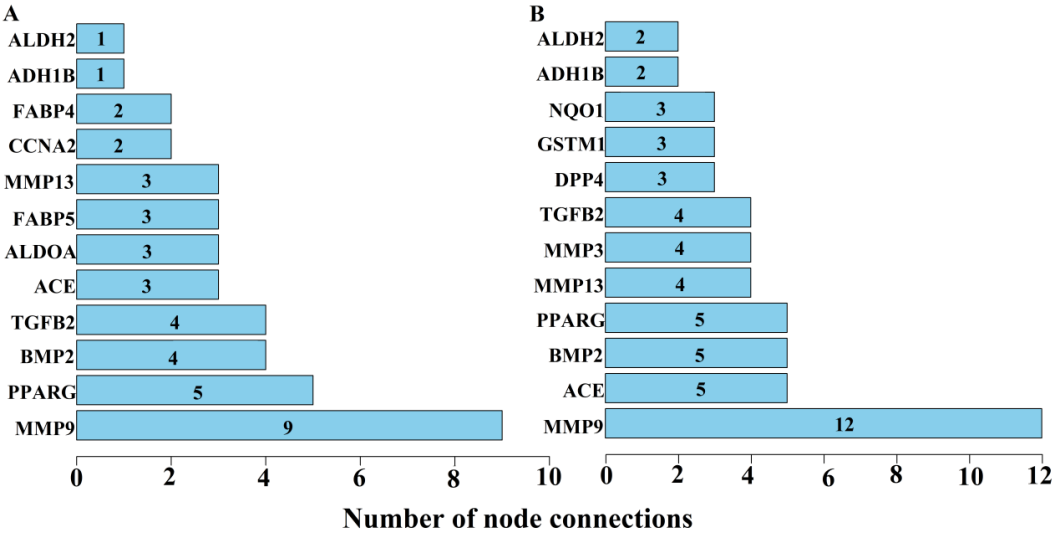


Fig. 5.

The number of node connections of major proteins in the protein-protein interaction network. A: The number of major protein node connections in the PPI network of periplogenin and LUAD. B: The number of major protein node connections in the PPI network between periplogenin and LUSC.

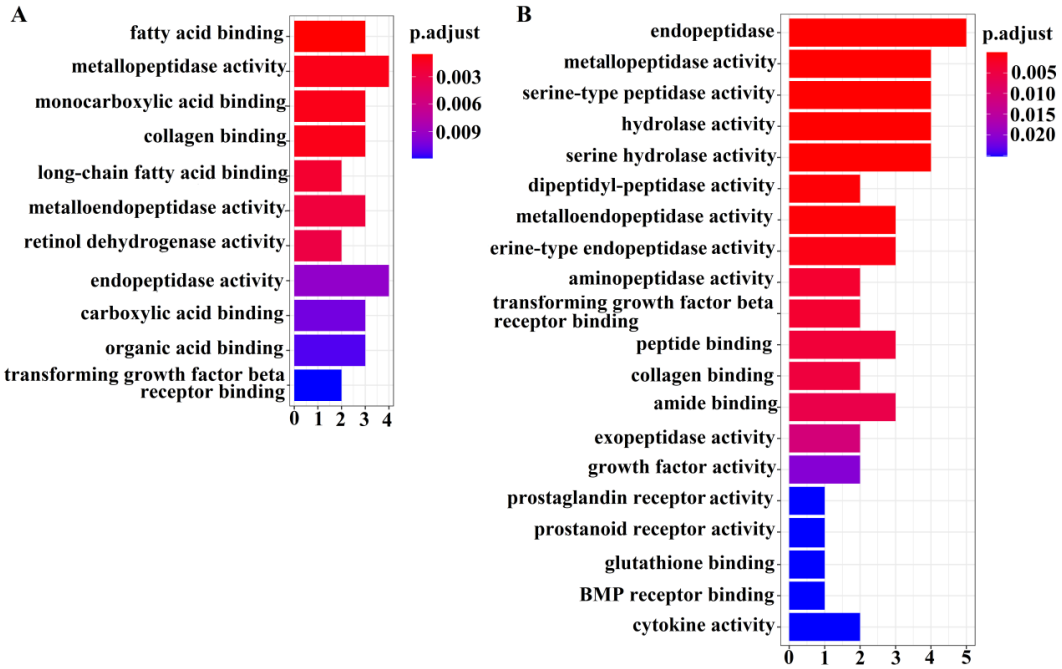


GO Function and KEGG Pathway Analysis

The results revealed that periplogenin was enriched by fast acid binding, metallopeptidase activity, monocarboxylic acid binding, collagen binding, long chain fatty acid binding, metalloendopeptidase activity, retinol dehydrogenase activity, carboxylic acid activity binding, organic acid binding, and transforming growth factor β receptor binding, and these biological functions act on LUAD (Fig. 6A). The results revealed that periplogenin mainly acted through endopeptidase activity, metallopeptidase activity, serine-type peptidase activity, and hydrolase activity, and these biological functions of acting on acid phosphorus nitrogen bonds, serine hydrolase activity, metalloendophytase activity, serine-type endophytase activity, peptide binding, amine binding, dipeptidyl peptidase activity, and aminopeptidase activity act on LUSC (Fig. 6B).

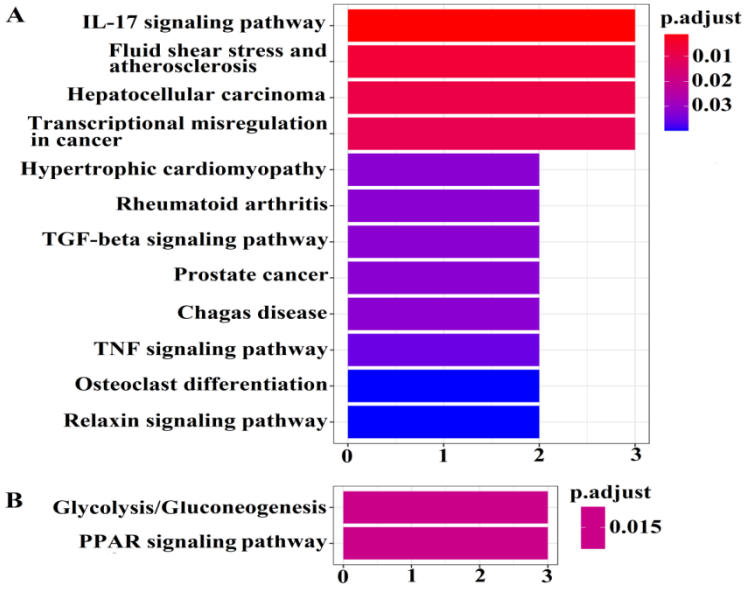
Fig. 6.

Functional enrichment analysis of GO. A: Main biological functions of periplogenin on LUAD. B: Main biological functions of periplogenin on LUSC.



The KEGG pathway was conducted on all intersection targets of periplogenin and LUSC, and no significant enriched signal pathway was found. However, a KEGG pathway enrichment analysis was performed for the MMP9, ACE, BMP2, PPARG, MMP13, MMP3, TGFB2, DPP4, GSTM1, NQO1, and IL-17 signaling pathways, and fluid shear stress, atherosclerosis, hepatocellular carcinoma, transcribed modulation in cancer, hypertrophic cardiopathy, rheumatoid arthritis, the transforming growth factor TGF-β signaling pathway, prostate cancer, Chagas disease, the TNF-signaling pathway, osteoclast differentiation, and the relaxin-signaling pathway were revealed to be the main signaling pathways of periplogenin acting on LUSC (Fig. 7A). The KEGG pathway enrichment analysis was performed on all intersection targets of periplogenin and LUAD, and two signal pathways with significant enrichment were identified (Fig. 7B).

Fig. 7.
KEGG enrichment pathway. A: Enrichment pathway of the intersection target of periplogenin and LUSC. B: Enrichment pathway of the intersection target of periplogenin and LUAD.



Discussion

Lung cancer is classified into two types of cancer: non-small cell carcinoma and small cell carcinoma. The proportion of non-small cell lung cancer (NSCLC) proportion is more than 80% of the total incidence of lung cancer mainly consists of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, with the two cell carcinomas being the main subtypes of NSCLC. Squamous cell carcinoma is large and pleomorphic, with abundant cytoplasm and tendency toward keratosis. Most of these cancers originated from segmental and subsegmental bronchi and tend to grow into the lumen of the trachea, which often leads to tracheal stenosis, atelectasis, and obstructive pneumonia in the early stage. In addition, cancer tissue is prone to necrosis and cavity. Adenocarcinoma is a more common type of lung cancer with irregular cells, obvious nucleoli, abundant cytoplasm, and mucus. Adenocarcinoma local infiltration through blood metastasis occurs early and easily involves the pleura, which causes pleural effusion. The pathological type and clinical stage of lung cancer treatment should be clear so that a comprehensive evaluation of the overall state of patients can be made. From this evaluation, an optimum treatment plan can be selected from the various available treatment methods so that the patient's symptoms can be reduced, their quality of life improved, and their survival period prolonged. Surgery is the first-line treatment for lung cancer, and it is suitable for most lung cancer patients in the early and middle stages and a few in the advanced stage. Of the patients who are in the advanced stage, 75% cannot undergo traditional surgery. Alternatives to traditional surgery include combined treatments of chemoradiotherapy, molecular targeted therapy, or minimally invasive surgery. More than 90% of lung cancer patients need chemotherapy. Approximately 1% of early-stage lung cancer patients can be cured by chemotherapy, except in NSCLC patients, for whom the best outcome of treatment is to relieve adverse symptoms and improve quality of life. At present, some progress has been made in chemotherapeutic drugs, such as the

implementation of nano-carrier modification, but these new treatments are expensive and they have not been popularized, so the overall survival rate of patients has not improved [11-13]. Therefore, the discovery of a novel drug that is cost-effective and provides a potential cure for lung cancer is still in urgent demand in clinical practice.

Periplogenin was extracted from cortex Periplocae and exhibited strong cytotoxicity. In our previous study, we found that periplogenin has a toxic effect on lung cancer cells, but its specific mechanism was not clear. Moreover, for common LUAD and lung squamous cell carcinoma (LUCS), it is not clear whether the effect and mechanism of periplogenin are the same. Network pharmacology is based on biology, and it analyzes the biological system network to select signal nodes that can be designed into drug molecules, which is then used in a multi-targeted approach. It is helpful to excavate mechanism of periplogenin and lung cancer and its related signal pathways. Therefore, this study, which is based on network pharmacology and molecular biology, has partly explored the mechanism of periplogenin in lung cancer treatment.

In this study, the interaction between periplogenin and LUAD, and periplogenin and LUSC were analyzed separately, and results displayed that MMP9, PPARG, BMP2, TGFB2, ACE, ALDOA, FABP5, MMP13, CCNA2, and FABP4 were the main targets of periplogenin acting on LUAD, and MMP9, PPARG, BMP2, and TGFB2 were the core proteins of periplogenin acting on LUAD. MMP9, ACE, BMP2, PPARG, MMP13, MMP3, TGFB2, DPP4, GSTM1, NQO1, ADH1B, ALDH2, ALDOA, CCNA2, KIT, RARA, CBR1, FABP4, KIF11, MMP12, PDE5A, and RARG are the main targets of periplogenin acting on LUSC. MMP9, ACE, BMP2, PPARG, MMP13, MMP3, and TGFB2 are the core proteins of periplogenin acting on LUSC.

As a family of zinc-dependent endopeptidases, matrix metalloproteinases (MMPs) have been detected in more than 26 subtypes in the human body. Matrix metalloproteinases (MMPs) can degrade and modify most components of the extracellular matrix and basement membrane, which are important for tumor invasion and metastasis [14]. Among MMPs, MMP-9 located on human chromosome 20q12-13 (MMP-9) is one of the most important enzymes that decompose the extracellular matrix. As a member of the MMPs family, MMP-9 is important in various types of cancer. Studies have shown that MMP-9 can degrade the extracellular matrix, and its overexpression can enhance the ability of tumor cells break through the basement membrane, thus promoting tumor invasion and metastasis [15, 16]. Some studies have shown that the expression of MMP-9 is correlated with lung cancer [17]. The MMP9 inhibitor can significantly stop the invasion and migration lung cancer cells via the ALDOA-hif-1 α axis [18]. Matrix metalloproteinase 13 (MMP13), also known as collagenase, is involved in the degradation of types I, II, III and VII collagen fibers and rapid remodeling of the extracellular matrix. In addition, MMP13 affected transcriptional activity [19, 20]. In recent years, the polymorphism of MMP-13 has produced contradictory results with various cancer risks [21, 22]. It can be concluded that periplogenin may act on lung cancer through MMP9, MMP13, and MMP3 in the MMP family.

PPARG encodes the receptor peroxisome proliferator-activated receptor (PPAR). PPAR forms heterodimers with retinoic acid X receptors that adjust the gene transcription. PPARG is involved in the regulation of myocardial ischemia/reperfusion, hypoxia/reoxygenation, induced apoptosis, oxidative stress, and myocardial cell membrane fluidity changes [23]. Bone morphogenetic protein-2 (BMP-2) belongs to the TGF family. *In vitro* experiments revealed that BMP-2 can induce epithelial–mesenchymal transition in various malignant tumor cells and participate in tumor invasion and metastasis [24]. The level of serum BMP-2 of lung cancer patients is higher than that of patients with non-distant metastasis, and it is positively correlated with the disease stage of lung cancer [25]. The TGFB family is the most important pathway of epithelial-mesenchymal transition (EMT). TGFB have dual antagonistic effects in tumorigenesis and development, can inhibit the growth of precancerous cells, and promote tumor invasion and metastasis through multiple mechanisms such as EMT [26]. The ACE, also known as kininogenase II or peptidyl carboxypeptidase, is widely distributed in human tissues, among which the ACE activity of pulmonary capillary endothelial cells is highest. Yoshiji et al. discovered that ACEI inhibits tumor development and angiogenesis by inhibiting the AngII level and then VEGF [27]. Thus, periplogenin can act on LUCS and LUAD through the above target genes.

In this study, GO and KEGG were used to analyze the above targets. The results revealed that periplogenin mainly passed through fatty acid binding, metallopeptidase activity, monocarboxylic acid binding, collagen binding, long chain fatty acid binding, metalloendopeptidase activity, retinol dehydrogenase activity, endopeptidase activity, carboxylic acid binding, organic acid binding, and TGF-β. In addition, these functions are mainly related to biological activity, Biological functions such as acting on acid phosphorus nitrogen bonds, serine hydrolase activity, metalloendopeptidase

activity, serine-type endophytase activity, peptide binding, amine binding, dipeptidyl peptidase activity, and aminopeptidase activity act on LUSC. Among the signal pathways enriched by KEGG, the IL-17-signaling pathway is the most abundant. Studies have shown that [28], lactic acid, a metabolite of the tumor hypoxia microenvironment, can upregulate the expression of IL-17. Thus, IL-17 in tumor tissue and peripheral blood of lung cancer patients is also significantly increased, and the high expression of IL-17 is associated with poor prognosis of patients, which suggests that IL-17 can promote the occurrence and progress of lung cancer. One of its possible mechanisms for promoting the progress of lung cancer is to promote tumor angiogenesis [29]. Transcriptional dysregulation in cancer is an important pathway by which periplogenin acts on LUSC. Whether DNA or non-coding RNA, its transcription process plays an important role in promoting or inhibiting tumor angiogenesis. As an endogenous cell growth inhibitor, TGF-β is usually a suppressor at the initial stage of lung cancer. However, if any link of the TGF-β signal transduction pathway fails in this stage, it may lead to abnormal signal transduction. Induction of growth inhibition and the apoptosis signal is not sensitive; it will appear to be cell proliferation or out of control differentiation and will cause lung cancer [30]. There are also signal pathways such as the TNF-signaling pathway, through which periplogenin may play a role in LUCS.

The KEGG pathway enrichment was analyzed for all the intersection targets of periplogenin and LUAD, and two signal pathways with significant enrichment were obtained, namely glycolysis/gluconeogenesis, and the PPAR-signaling pathway. Glucose metabolism of cancer cells mainly depends on glycolysis rather than the tricarboxylic acid cycle. Since the rapid growth of cancer cells will consume a large amount of glucose, the mechanism can continuously provide glucose for cancer cells through the reverse reaction of glycolysis, namely gluconeogenesis [31]. In this study, we found that the glycolysis/gluconeogenesis pathway is the main pathway by which periplogenin acts on LUAD. Therefore, periplogenin may inhibit LUAD by inhibiting gluconeogenesis. PPARs belong to the internal receptor superfamily and are expressed in many tissues, such as adipocytes, hepatic parenchymal cells, skeletal muscles, and endothelial cells. There are three subtypes, PPAR-α, PPAR-β, and PPAR-γ. At present, PPAR-γ has received great attention in clinical and laboratory studies, and it is the most widely studied subtype. It not only plays a considerable role in the process of fat synthesis and energy metabolism, but it also participates in the development of malignant tumors. After being activated by agonists, it can inhibit proliferation and promote apoptosis of tumor cells through the molecular-signaling pathway. It can also affect the angiogenesis of macrophages and tumors, and potentially delay tumor development. The related ligands may become the new therapy of tumors by changing the pathways that promote tumor development and its microenvironment [32]. Therefore, periplogenin may inhibit lung cancer cells by activating the PPAR-signaling pathway.

CONCLUSION

MMP9, ACE, BMP2, PPARG, MMP13, MMP3, and TGFB2 are the core proteins of periplogenin acting on LUAD. Through a GO enrichment analysis of intersection targets, it was found that periplogenin mainly acted on LUAD through fatty acid binding, metallopeptidase activity, and monocarboxylic acid binding. Periplogenin mainly acts on LUSC through endopeptidase activity, metallopeptidase activity, and serine-type peptidase activity. After KEGG enrichment analysis, the IL-17-signaling pathway, fluid shear stress and atherosclerosis, hepatocellular carcinoma, transcriptional misregulation in cancer, hypertrophic cardiopathy, rheumatoid arthritis, TGF-β-signaling pathway, prostate cancer, Chagas disease, TNF-signaling pathway, osteoclasts were obtained. Differentiation and the relaxin-signaling pathway are the main signaling pathways of periplogenin acting on LUSC, and glycolysis/gluconeogenesis and the PPAR-signaling pathway are the main signaling pathways of periplogenin acting on LUAD. This shows that periplogenin in lung cancer treatment (including LUCS and LUAD) is through a multi-target, multi-channel approach.

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