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Study on the mechanism of anlotinib in the treatment of non-small cell lung cancer based on network pharmacology and molecular docking technology

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Author contributions

Qiang Wu formulated the research objectives and designed this study, and was responsible for writing the paper. Yu-Ting Zhu was responsible for data analysis and data plotting. Leng-Qiu Guo was responsible for data collection, and Rui Liang was responsible for manuscript editing.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

EGFR, epidermal growth factor receptor; MAPK14, mitogen-activated protein kinase 14; HSP90AA1, heat shock protein HSP 90-alpha; LCK, tyrosine-protein kinase Lck; PRKACA, cAMP-dependent protein kinase catalytic subunit alpha; NSCLC, non-small cell lung cancer; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Anl, Anlotinib; PPI; protein-protein interaction.

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Abstract

Objective: Based on the analysis of a biochemical information database, the "target-pathway" network of anlotinib in the treatment of non-small cell lung cancer was constructed by using network pharmacological methods to explore the mechanism of multi-target and multi-pathway treatment of non-small cell lung cancer. **Methods:** The 3D molecular structure formula of anlotinib was obtained by searching the PubChem database, and the target of anlotinib was predicted by using the PharmMapper database; obtain non-small cell lung cancer related targets through the GeneCards database, screen common genes related to drug targets and diseases by Venny 2.1.0, and build the relationship between drugs and diseases. Through the STRING11.5 database, the interaction relationship between action targets was built, the protein-protein interaction network was constructed, and the target degree was analyzed by Cytocsape 3.7.2 software to screen molecular docking objects. The DAVID database was used for Gene Ontology gene enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis to predict its mechanism, and the AutoDock software was used for molecular docking of the main targets. **Results:** The analysis results showed that there were 76 possible targets involved in the treatment of non-small cell lung cancer with anlotinib, mainly acting on epidermal growth factor receptor, mitogen-activated protein kinase 14, tyrosine-protein phosphatase non-receptor type 11, heat shock protein HSP 90-alpha, tyrosine-protein kinase Lck, cAMP-dependent protein kinase catalytic subunit alpha and other target protein genes, Kyoto Encyclopedia of Genes and Genomes pathway analysis obtained 60 possible pathways related to its treatment of non-small cell lung cancer, mainly involving progesterone-mediated oocyte maturation, prostate cancer, proteoglycans in cancer, FoxO signaling pathway, pathways in cancer, Ras signaling pathway, PI3K-Akt signaling pathway, etc. **Conclusion:** Anlotinib has the characteristics of multi-targets and multi-pathways in the treatment of non-small cell lung cancer, which provides a scientific basis for the follow-up study on the optimization of its efficacy in the treatment of non-small cell lung cancer and the revelation of the pharmacological effects of anlotinib.

Keywords: non-small cell lung cancer; anlotinib; network pharmacology; EGFR; molecular docking

Introduction

Lung cancer accounts for the majority of deaths from malignant tumors, and about 6.102 million people die of lung cancer every year in China. Most of them are non-small cell lung cancer (NSCLC). Lung cancer is typically diagnosed as advanced, and the survival rate is low [1]. In further biological research of NSCLC, a new angiogenesis inhibition therapy was developed. Preclinical studies are exploring the benefits of using angiogenesis inhibitors. Raben outlined these preclinical studies and the rationale of this treatment strategy in the treatment of NSCLC [2]. Aftab characterized the anti-angiogenesis and anti-cancer activity of itraconazole in preclinical models related to angiogenesis and lung cancer [3]. VEGF-Trap plays an anti-angiogenesis role by regression of tumor vasculature, remodeling or normalization of survival vasculature, and inhibition of new tumor angiogenesis. Rugamba investigated the impact of VEGF inhibition on the clinical results of NSCLC patients, including the efficacy of ICI treatment [4].

Anlotinib (Anl, PubChem CID: 25017411, Figure 1) is a newly developed oral small molecule tyrosine kinase inhibitor. Developed by China Jiatai Tianqing Pharmaceutical Corporation [5]. As a multi-target receptor RTK inhibitor, it mainly acts on vascular endothelial growth factor receptor, fibroblast growth factor receptor and other targets: that is, it mainly acts on angiogenesis related targets [6]. Therefore, it can be speculated that Anl has a broad-spectrum inhibitory effect on angiogenesis and malignant tumor growth and can be used for the treatment of advanced NSCLC.

Materials and methods

Databases retrieval

The databases involved in this study include: PubChem (https://pubchem.ncbi.nlm.nih.gov/), PharmMapper (http://www.lilab-ecust.cn/pharmmapper/), UniProt (https://beta.uniprot.org/), GeneCards (https://www.genecards.org/), CTD (http://ctdbase.org/), DAVID (https://david.ncifcrf.gov/), STRING11.5 (https://www.string-db.org/), Cytoscape3.7.2 (https://cytoscape.org/), Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html), Pymol (https://pymol.org/2/), OpenBabel (http://openbabel.org/wiki/Main_Page), RCSB PDB (https://www.pdbus.org/), image GP (http://www.ehbio.com/ImageGP/).

Structure acquisition of Anl compounds

The PubChem database was used to obtain the 3D molecular structure formula of Anl which was stored in *.sdf format.

Prediction of the active target of Anl

Uploading the Anl.sdf to the PharmMapper database, the 'Select Targets Set' option selects the 'Human Protein Targets' option, and the other options are the default options for analog molecule target docking. Furthermore, the 'UniProt ID' of the target is transformed into the 'Gene Name' through the UniProt database.

NSCLC target screenings

Search 'Non-Small Cell Lung Carcinoma' through GeneCards and CTD databases to obtain NSCLC disease-related target data.

Common target screenings

The obtained Anl target genes and NSCLC target genes were mapped to screen the common genes, so as to obtain the Anl target for NSCLC treatment.

Construction of the protein interaction network

The protein-protein interaction network (PPI) was constructed on the String11.5 platform database for the selected NSCLC target proteins of Anl treatment [7]. The species was set as human, and the lowest interaction score was higher than 0.9. The target protein interaction relationship was obtained. Isolated items were deleted, and the protein connection was selected to indicate the protein interaction. The closer the connection was, the higher the protein correlation was, and the protein interaction network was obtained. Then, the protein interaction network was analysed by using the software Cytoscape 3.7.2 to obtain the corresponding list of node-degree trees [8].

Molecular docking verifications

Analyse the obtained PPI network topology parameters (degree), screen out core targets, and use AutoDock Vina software to verify the molecular docking with Anl [9].

GO function and KEGG signal pathway enrichment analysis

The DAVID database was used to perform Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway enrichment analysis on common targets. The 'Select identifier' was set to 'official gene symbol', the 'List type' was set to 'gene list', and the species was limited to 'homo sapiens'. The biological function and main signaling pathway of the NSCLC targets treated with Anl were analyzed. The 'Functional Annotation Chart' was selected to download data, and the results were output as bubble charts through the image GP website.

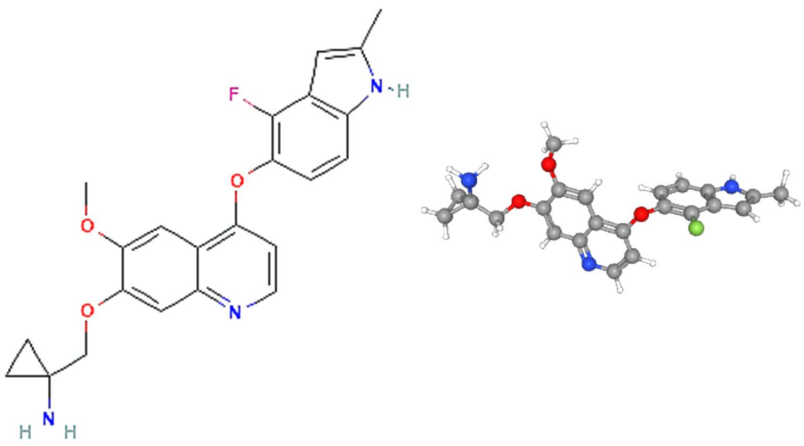

Figure 1 Chemical structure of androtinib. Based on network pharmacology and molecular docking methods, this study took Anl as the research object and comprehensively predicted the action targets of Anl in the treatment of NSCLC by establishing a "target-pathway" network to explain its possible therapeutic mechanism, providing support for further revealing the action mechanism of Anl and designing improved drugs for Anl. Anl, Anlotinib; NSCLC, non-small cell lung cancer.

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Results

Prediction of the active target of Anl

With the results given by the PharmMapper database, 'Z'-score' has better statistical significance and confidence interval than 'Fit score' and is the same as 'Fit score'. The higher the Z'-score is, the better the combination of compound molecules and protein targets [10]. Therefore, Z'-score higher than 0.5 was selected as an effective target, 112 target proteins were screened from the docking results of PharmMapper molecule target proteins, the UniProt ID was converted into Gene Name through UniProt database, and the results were checked to eliminate duplicate genes, resulting in a total of 111 target genes.

Common targets of Anl and NSCLC

The predicted Anl target, 5304 (GeneCards) and 23087 (CTD) NSCLC related genes mined from the GeneCards and CTD databases were used to construct a Venn map of the common target through the Venny2.0 mapping platform, as shown in Figure 2. The intersection is the common target of Anl-NSCLC. 76 therapeutic targets of Anl for NSCLC and their tumor correlation scores in the GeneCards database were screened, as shown in Table 1.

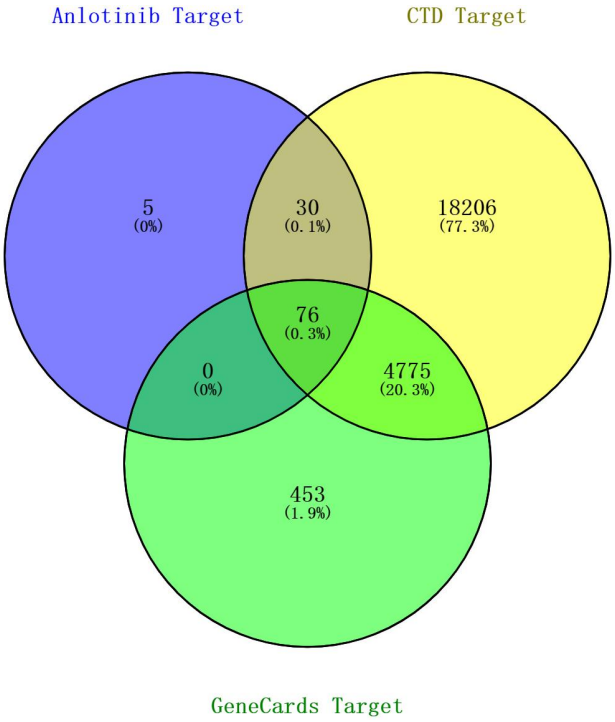

Figure 2 Intersection of Anl Related Targets and Anti-NSCLC Targets. Anl, Anlotinib.

Table 1 Screening Results of Action Targets of Anl in the Treatment of NSCLC

Pharma Model	Z'-score	Gene Name	Score
1h6g_v	2.59099	CTNNA1	44.13
1a28_v	2.50829	PGR	58.62
1svh_v	2.50719	PRKACA	34.18
3f5p_v	2.38966	IGF1R	68.72
1utt_v	2.36106	MMP12	33.08
1xan_v	2.30603	GSR	19.42
1hvy_v	2.30199	TYMS	52.4
3bbt_v	2.15531	ERBB4	54.2
1kak_v	2.11757	PTPN1	25.88
1xwk_v	2.07175	GSTM1	58.43
1fm6_v	2.05573	PPARG	78.78
1imx_v	1.95857	IGF1	57.1
1iz2_v	1.74998	SERPINA1	52.44
2shp_v	1.74566	PTPN11	72.89
1mrq_v	1.74435	AKR1C1	27.21

Table 1 Screening Results of Action Targets of Anl in the Treatment of NSCLC (*continued*)

Pharma Model	Z'-score	Gene Name	Score
1qyx_v	1.7226	HSD17B1	29.21
1wma_v	1.65019	CBR1	11.88
1lt8_v	1.63307	BHMT	7.56
2gpq_v	1.59066	EIF4E	40.47
1dxo_v	1.59046	NQO1	49.92
1og5_v	1.54096	CYP2C9	11.67
2bu5_v	1.51844	PDK2	10.37
1s19_v	1.5061	VDR	37.16
2ity_v	1.46197	EGFR	312.61
1r1h_v	1.39574	MME	46.55
1uhl_v	1.38957	NR1H3	13.44
1qab_v	1.3733	RBP4	15.92
1lv2_v	1.35646	HNF4G	6.8
1fkg_v	1.35615	FKBP1A	13.54
1nuo_v	1.34588	THRB	15.14
1t46_v	1.34318	KIT	122.82
1hov_v	1.26938	MMP2	78.11
1cg6_v	1.2314	MTAP	20.17
2qd9_v	1.20699	MAPK14	58.13
1dkf_v	1.16961	RARA	50.59
1uym_v	1.09475	HSP90AB1	19.96
2oi0_v	1.07779	ADAM17	26.88
1d3g_v	1.06646	DHODH	7.71
2pjl_v	1.06244	ESRRA	14.9
1tfg_v	1.00525	TGFB2	38.75
1m48_v	0.992334	IL2	74.45
1gse_v	0.990211	GSTA1	15.12
2o9i_v	0.958568	NR1I2	17.47
1xap_v	0.888147	RARB	62.04
1wda_v	0.876494	PADI4	12.85
1qcf_v	0.856118	HCK	22.14
2p4i_v	0.850624	TEK	38.42
1s9j_v	0.847951	MAP2K1	128.54
2opy_v	0.840739	XIAP	62.67
2roy_v	0.791023	TTR	22.24
1xf0_v	0.786566	AKR1C3	18.02
2irw_v	0.782511	HSD11B1	13.08
2hmb_v	0.771866	FABP3	11.42
2bz5_v	0.743864	HSP90AA1	51.12
2o65_v	0.711296	PIM1	24.35
830c_v	0.681693	MMP13	39.06

Table 1 Screening Results of Action Targets of Anl in the Treatment of NSCLC (*continued*)

Pharma Model	Z'-score	Gene Name	Score
1xil_v	0.663713	SOD2	43.97
2pir_v	0.656255	AR	57.39
1nhz_v	0.649	NR3C1	36.18
2hzi_v	0.645275	ABL1	59.38
1tbf_v	0.643684	PDE5A	14.11
2w1e_v	0.639022	AURKA	54.67
1j96_v	0.622768	AKR1C2	12.85
1rs0_v	0.614605	CFB	13.44
1p4f_v	0.614132	DAPK1	44.42
2pg2_v	0.604413	KIF11	13.61
2of2_v	0.601351	LCK	37.51
2a4z_v	0.597939	PIK3CG	51.55
1w8l_v	0.588996	PPIA	17.01
1j78_v	0.585225	GC	13.73
1kjr_v	0.567074	LGALS3	46.92
1h9u_v	0.560444	RXRB	30.65
1yvj_v	0.55761	JAK3	96.79
1shj_v	0.534232	CASP7	37.04
1gfw_v	0.527456	CASP3	82.44
1cbs_v	0.504313	CRABP2	17.04

Anl, Anlotinib; NSCLC, non-small cell lung cancer.

Protein interaction network analysis

76 therapeutic NSCLC target protein genes related to Anl were screened, and the target protein interaction network was obtained through STRING database (Figure 3). In the figure, nodes represent proteins, while edges represent associations between proteins. Use the Cytoscape 3.7.2 software to analyze the PPI network with Network Analyzer, and calculate the degree value of the node target protein. The higher the degree value, the more important the target protein is. The analysis of network topology structure shows that the co-expression network clustering correlation coefficient is 0.384, the number of network nodes (target genes) is 51, produce effects through 108 edges, and the average number of adjacent nodes is 4.196, indicating that Anl treating NSCLC has a multi-target attribute, and there is a strong correlation between target proteins.

Molecular docking analysis

According to the above network analyzer analysis results, select the target whose degree topology parameter value is greater than twice the median (degree = 4) as the core target, and download its target protein file (*. pdb) through the RCSB PDB database, and remove water molecules and ligands through PyMol software for storage. Convert the Anl.sdf file to *.mol2 file through OpenBabel software. Then AutoDock Vina software was used to perform molecular docking between the processed protein file and ligand file to further confirm the binding activity of these 6 targets with Anl, as shown in Table 2. It is generally believed that the lower the maximum binding energy of proteins with small molecules, the stronger the molecular binding, indicating that proteins and molecules are easier to bind. The results show that Anl has 6 core targets. It shows that Anl has good binding activity with MAPK14, PTPN11, EGFR, HSP90AA1, LCK and PRKACA (molecules and proteins are generally bound by hydrogen bonds, and the bond energy is generally 2–8 Kcal/mol, so it can be considered that the maximum binding energy in the docking results is greater than −5 Kcal/mol, indicating that they have good binding activity [11]); At the same time, it can be found from Table 1 that the 6 core targets also have good anti NSCLC correlation. Querying the CTD database, it can be found that there are 22 related compounds at MAPK14 target, which have greater similarities with the more representative compound molecules (5 - [(4-Methylphenyl) methyl] - 2 - (phenylamino) - 4 (5h) - thiazolone, PubChem CID: 135580376). Using the same method, query PTPN11, EGFR, HSP90AA1, LCK PRKACA obtained that PTPN11 has 11 related compounds, which are quite similar to gemcitabine (PubChem CID: 60749), EGFR has 37 related compounds, which are quite similar to Canertinib (PubChem CID: 156414), HSP90AA1 has 18 related compounds, which is quite similar to Gefitinib (PubChem CID: 123631), LCK has 10 related compounds, which is quite similar to Crisotinib (PubChem CID: 11626560), and PRKACA has 7 related compounds, which are quite similar to sulindac sulfide (PubChem CID: 5352624). In the above example drugs, under the 3D graphic display, the overall molecules are long chains, there is a carbon ring or carbon heterocycle in the center, and there are one or more carbon chains or carbon rings or carbon heterocycles on both sides. The heteroatoms on them may combine with the target in the form of hydrogen bonds. Therefore, according to the theory of drug structure activity relationships, it can be proven that Anl may have similar drug activity to the known compounds on the above target, The presence of three tinib compounds in the relevant compounds can also prove that the above targets may be the core targets for the treatment of NSCLC. The reliability of PharmMapper target prediction is further verified.

GO function and KEGG signal pathway enrichment analysis

76 NSCLC targets were input into the DAVID database for GO analysis, including biological process, cellular component and molecular function. The GO results showed that 184 biological processes, 31

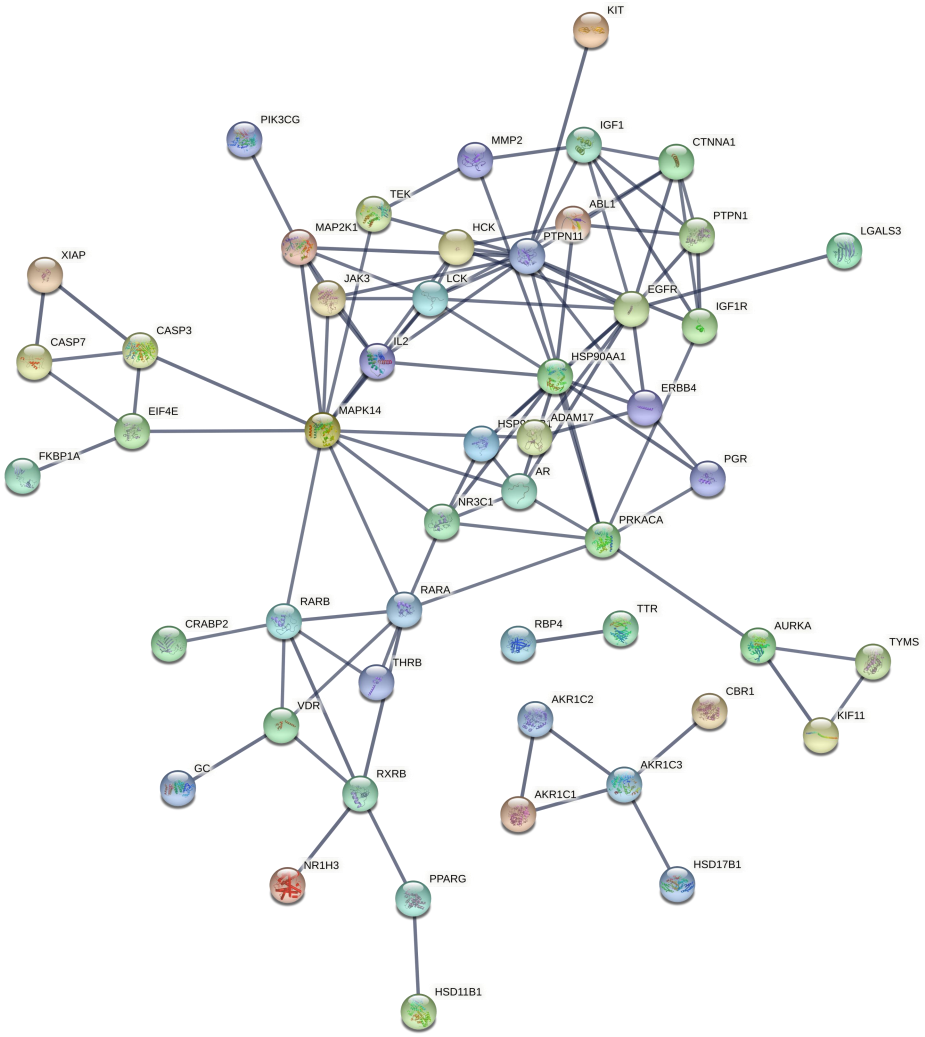

Figure 3 Protein-protein interaction core networks of NSCLC treated with Anl. Anl, Anlotinib; NSCLC, non-small cell lung cancer.

Table 2 Molecular Docking Results of Core Targets of NSCLC Treated with Anl

No	PDB ID	Gene Name	Protein names	Degree	Maximum binding energy (Kcal/mol)
1	2qd9	MAPK14	Mitogen-activated protein kinase 14	13	−7.4
2	2shp	PTPN11	Tyrosine-protein phosphatase non-receptor type 11	13	−8.2
3	2ity	EGFR	Epidermal growth factor receptor	12	−7.6
4	2bz5	HSP90AA1	Heat shock protein HSP 90-alpha	11	−8.3
5	2of2	LCK	Tyrosine-protein kinase Lck	9	−8.0
6	1svh	PRKACA	cAMP-dependent protein kinase catalytic subunit alpha	8	−7.2

Anl, Anlotinib; NSCLC, non-small cell lung cancer.

cellular processes and 75 molecular functional processes were enriched. According to the P value, the first 10 items are visualized to form a bubble chart (Figure 4). The results showed that Anl may participate in the regulation of the steroid hormone mediated signaling pathway, transcription initiation from the RNA polymerase II promoter, protein autophosphorylation, negative regulation of apoptotic process, positive regulation of cell proliferation, peptidyl-tyrosine phosphorylation, phosphatidylinositol-mediated signaling, signal transduction, the intracellular receptor signaling pathway, peptidyl-tyrosine autophosphorylation and other biological process, cellular component and molecular function play a role in the treatment of NSCLC. KEGG results showed that 60 KEGG pathways were obtained, and the top ones were: cancer pathways in cancer, progesterone-mediated oocyte maturation, proteoglycans in cancer, metabolism of xenobiotics by cytochrome P450, Ras signaling pathway, FoxO signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, estrogen signaling pathway, non-small cell lung cancer pathway, steroid hormone biosynthesis, and Rap1 signaling pathway. It is speculated that Anl may play a role in the treatment of NSCLC by regulating the above signal pathways. According to the P value, the top 20 results will form a bubble diagram of KEGG function enrichment (Figure 5). According to the analysis of the number of pathway genes involved, P value and enrichment factor, the original 7 pathway related gene targets are shown in Table 3.

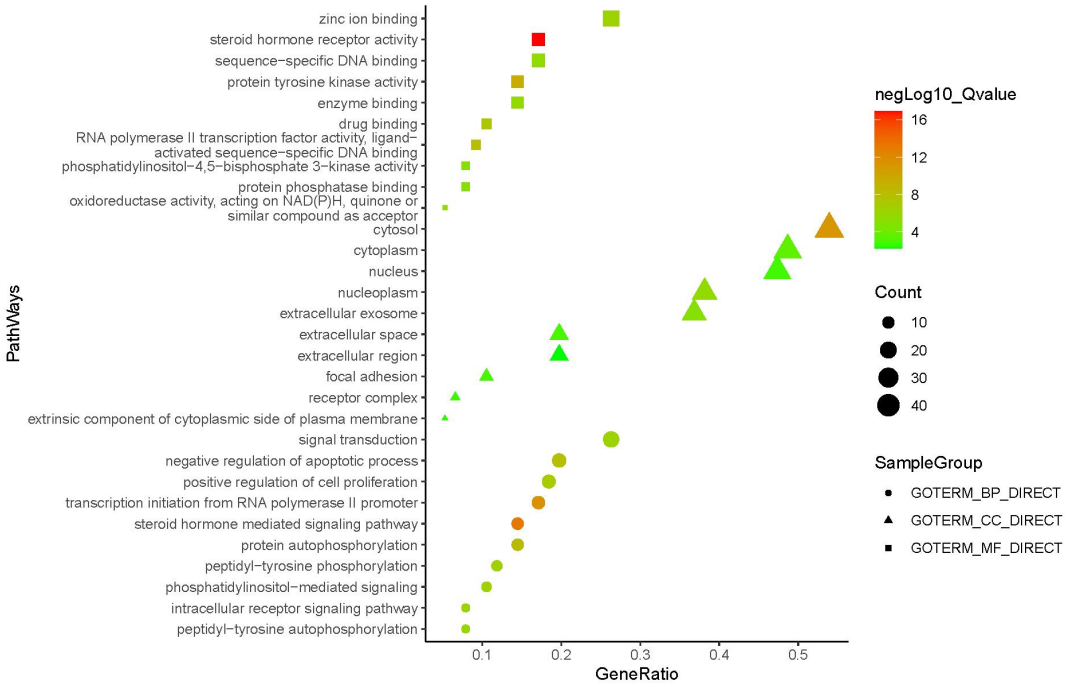

Figure 4 GO Function Enrichment Analysis Diagram. GO, Gene Ontology.

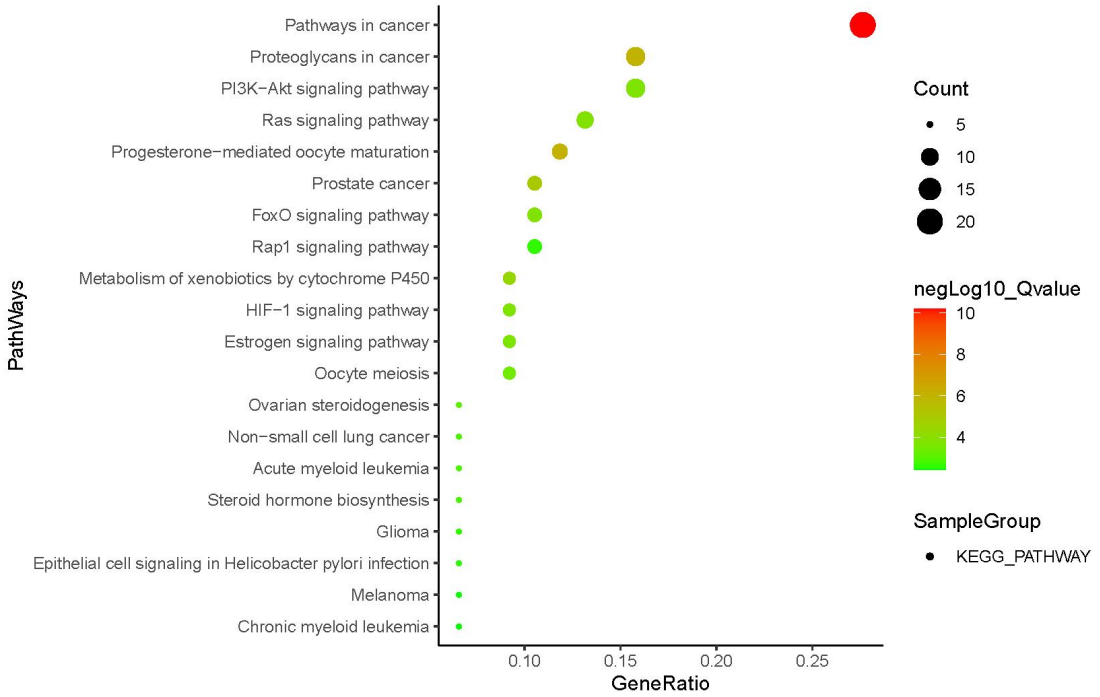

Figure 5 Enrichment Analysis of KEGG Signal Pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 3 List of gene targets related to the first 7 pathways

No	Pathway	Count	Pvalue	Genes
1	Progesterone-mediated oocyte maturation	9	8.06E–07	MAP2K1,HSP90AA1,HSP90AB1,PGR,IGF1,MAPK14,PRKACA,PIK3CG,IGF1R
2	Prostate cancer	8	1.10E–05	AR, MAP2K1, HSP90AA1, HSP90AB1, IGF1, EGFR, PIK3CG, IGF1R
3	Proteoglycans in cancer	12	1.12E–06	TGFB2, MAP2K1, ERBB4, CASP3, MMP2, PTPN11, IGF1, MAPK14, PRKACA, EGFR, PIK3CG, IGF1R
4	FoxO signaling pathway	8	1.65E–04	TGFB2, MAP2K1, IGF1, MAPK14, SOD2, EGFR, PIK3CG, IGF1R
5	Pathways in cancer	21	6.08E–11	TGFB2, MAP2K1, HSP90AA1, HSP90AB1, DAPK1, MMP2, XIAP, IGF1, EGFR, PIK3CG, IGF1R, RXRB, AR, CASP3, KIT, CTNNA1, ABL1, RARA, RARB, PPARG, PRKACA
6	Ras signaling pathway	10	1.49E–04	MAP2K1, KIT, ABL1, PTPN11, TEK, IGF1, PRKACA, EGFR, PIK3CG, IGF1R
7	PI3K-Akt signaling pathway	12	1.88E–04	MAP2K1, HSP90AA1, HSP90AB1, KIT, TEK, IGF1, JAK3, EIF4E, EGFR, IL2, PIK3CG, IGF1R

Discussion

In the research on the treatment of NSCLC with Anl, most of the studies focused on the clinical effect of Anl and the discovery of the predictors of Anl [6, 12]. In this study, through the use of network pharmacology, molecular docking and other methods, the database was used to analyze and explore the possible mechanism of action of Anl, and provide a basis for the design of improved drugs for Anl.

In the core pathway, MAPK14 participated in proteoglycans in cancer, the FoxO signaling pathway, etc. Jia provided evidence that the MAPK14 rs3804451 G > A variant may regulate the survival results of patients with advanced NSCLC treated with first-line platinum chemotherapy [13]. Chemotherapy is often used in combination with drugs such as Anl in clinical practice to achieve better therapeutic effects. This study may explain the theoretical basis of this clinical therapy, that is, both can regulate gene expression through MAPK14 at the same time to achieve better therapeutic effects. PTPN11 has participates in proteoglycans in cancer, the Ras signaling pathway, etc. There are few studies on the PTPN11 gene, so there is no relevant study to prove its effect in NSCLC treatment; EGFR is involved in prostate cancer, proteoglycans in cancer, the FoxO signaling pathway, pathways in cancer, the Ras signaling pathway, the PI3K-Akt signaling pathway, etc. EGFR is more common in advanced NSCLC, and is often shown to reduce drug reactivity due to EGFR mutation, Kobayashi reported a case of EGFR mutation, reduced gefitinib reactivity, and advanced NSCLC patients [14]. The patient relapsed two years after complete remission during gefitinib treatment. A clinical trial using erlotinib and placebo in NSCLC showed that erlotinib had survival benefits. Tsao used tumor biopsy samples from the test participants to study whether the responsiveness to erlotinib and its impact on survival are related to the expansion and mutation of epidermal growth factor receptor and EGFR genes [15]. This study shows that the acquisition of drug resistance is related to specific secondary somatic mutation EGFR T790M. Relevant studies have shown that EGFR mutation plays an important role in the treatment of NSCLC. In the future, the improvement direction of tinib drugs can focus on how to still have a better therapeutic effect when EGFR receptor mutation occurs; HSP90AA1 participated in progesterone-mediated oocyte formation, prostate cancer, pathways in cancer, PI3K-Akt signaling pathway, etc. Liu studied the potential prognostic value of members of the HSP 90 family in patients with NSCLC and confirmed that HSP90AA1 and other HSP90 members may be potential drug targets and biomarkers for the treatment of NSCLC [16]. Bhattacharyya's research using network theory methods shows that CDK1 and HSP90AA1 are key regulatory genes in the complex NSCLC network [17]. To sum up, HSP90AA1 and other related targets may play a certain role in the treatment of NSCLC and can be used as biomarkers of efficacy to a certain extent; PRKACA has participated in progesterone-mediated oocyte formation, proteoglycans in cancer, pathways in cancer, Ras signaling pathway, etc. Glycolysis is one of the characteristics of cancer. It has been confirmed that the maladjusted modification of glycolytic enzyme family is phosphorylation: blocking TPI Ser58 phosphorylation can significantly inhibit glycolysis and prevent cancer growth, and metastasis, PRKACA protein kinase directly phosphorylates TPI Ser58 to enhance glycolysis and lead to cancer growth and metastasis [18]. Therefore, Anl may play a role in treating NSCLC by blocking TPI Ser58 phosphorylation as a PRKACA kinase inhibitor. To sum up, most of the core targets are involved in the activation of similar multiple core pathways to inhibit NSCLC.

To sum up, the treatment of NSCLC with Anl involves multiple biological processes and signaling pathways. The core targets of treatment are MAPK14, PTPN11, EGFR, HSP90AA1, LCK and PRKACA. The core signaling pathways are progesterone-mediated oocyte maturation, prostate cancer, proteoglycans in cancer, FoxO signaling pathway, pathways in cancer, Ras signaling pathway, PI3K-Akt signaling pathway, which indicates that Anl has a multi-target and multi-pathway mechanism in the treatment of NSCLC. At the same time, this article also has its own limitations and shortcomings. For example, computer simulation of molecular docking and target screening analysis belong to theoretical research, which is not enough to fully explain the pharmacological effect of Anl in the treatment of NSCLC. Therefore, cell and animal experiments are needed in the next research process to more fully explain the pharmacological mechanism of Anl in the treatment of NSCLC.

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