DOI: 10.53469/jcmp.2024.06(12).43

Exploration on the Mechanism of Fufei Jiedu Prescription in the Treatment of Lung Adenocarcinoma based on Network Pharmacology and Molecular Docking

Yali Chen*, Yuanchun Wang, Yuxian Wang, Jiejie Li, Peihua Shen

Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
**Correspondence Author*

Abstract: ***Objective:*** ***This investigation aims to elucidate the mechanism of action underlying the Fufei Jiedu formula in the treatment of lung adenocarcinoma by employing network pharmacology techniques and molecular docking strategies.*** ***Methods:*** ***The TCMSP, HERB, BATMAN, ETCM databases and analysis platforms were utilized to identify active ingredients and potential targets. The genes associated with lung adenocarcinoma were searched in the OpenTargets, DrugBank, and GeneCards databases to identify the intersection targets of the FuFei JieDu detoxification and lung adenocarcinoma. Subsequently, a FuFei JieDu detoxification-component-target-lung adenocarcinoma network model map was created using Cytoscape3.9.1 software. Additionally, The STRING database was utilized to construct a protein-protein interaction (PPI) network map in order to identify key targets. The GO function and KEGG pathway enrichment analyses were conducted to explore the potential mechanisms of drug action on the disease. Finally, molecular docking was conducted to confirm the binding affinity of key active components and core targets using AutoDock Tools software.*** ***Results:*** ***The research identifying 99 active compounds and their 582 associated targets connected to lung adenocarcinoma. 121 intersection targets were selected, including 5 chemical compound that serve as the material basis for the treatment of lung adenocarcinoma, luteolin, wogonin, baicalein and kaempferol. After analyzing the PPI network map, core targets such as TP53, AKT1, STAT3, EGFR, MYC, BCL2, and CTNNB1 were identified. The GO and KEGG enrichment analysis revealed that121 genes were primarily enriched in biological processes such as programmed cell death, immune regulation, and oxidative stress, as well as signaling pathways including PI3K/Akt, AGE/RAGE, TNF, and PDL-1pathway. The outcomes of the molecular docking studies uncovered these associations, compounds such as EGFR, CTNNB1, and AKT1 had good affinity with target molecules like quercetin, Calycosin, baicalin, β-sitosterol, and Delta-D.*** ***Conclusion:*** ***A variety of components such as quercetin, Calycosin, baicalin, β-sitosterol, and Delta-D in the Fufei Jiedu prescription may participate in the regulation of multiple pathways such asPI3K/Akt, AGE/RAGE, TNF, and PDL-1 pathway by acting on EGFR, CTNNB1, AKT1 and other targets to inhibit the proliferation, invasion and metastasis of lung adenocarcinoma, and play a synergistic anti-tumor effect.***

Keywords: Fufei Jiedu prescription, Lung adenocarcinoma, Network pharmacology, Molecular dockings**.**

1. Introduction

According to the International Agency for Research on Cancer (IARC) [1], there will be about 20 million new cases of cancer worldwide in 2022, of which about 2.5 million will be lung cancer, accounting for 12.4% of all newly diagnosed cancers in 2022. In the same year, the number of new cases and deaths from lung cancer in China accounted for 22.0% and 28.5% of all malignant tumors, respectively, ranking first among all malignant tumors [2]. The World Health Organization (WHO) classifies lung cancer into two main types: non-small cell lung cancer (SCLC) and small cell lung cancer (NSCLC). Among NSCLC subtypes, lung adenocarcinoma (LUAD) accounts for nearly 40% [3]. LUAD usually has an insidious onset and is highly metastatic, with a general progression of adenocarcinoma—situ-microinvasive adenocarcinoma—invasive adenocarcinoma. Due to technological limitations in early detection, many patients with LUAD have advanced disease at the time of their initial presentation, which seriously affects the outcome of LUAD treatment and patient survival [4]. Currently, the treatment strategy for advanced lung adenocarcinoma is based on systemic therapy, including radiotherapy, targeted therapy, immunotherapy and other methods [5]. Treatment of LUAD with mutations in driver genes such as EGFR, AKT, ROS1, RET, MET, BARF and NTRK is molecularly targeted therapy; In contrast, for advanced LUAD without detectable mutations in these driver genes, platinum-containing doublet chemotherapy or PD-1/PD-L1 inhibitor immunotherapy is the standard first-line treatment. Although molecular targeting and immunotherapy have continued to make new advances in the treatment of LUAD, providing some survival benefit to patients, But they often lead to treatment discontinuation and poor prognosis due to multiple adverse effects and the emergence of acquired resistance, with a 5-year survival rate of only 18% [6]. Therefore, major challenges remain in the clinical management and prognosis of LUAD. Due to its characteristic of “Treatment Determination Based on Syndrome DIfferentiation”, Chinese medicine plays a comprehensive regulatory role of multiple components, multiple targets and multiple pathways in improving the therapeutic efficacy of LUAD by reconstructing the tumor microenvironment and regulating the immune response, and has shown good results in the systemic treatment of lung cancer, which has become one of the important therapeutic means for lung cancer. Especially for those patients who are difficult to tolerate radiotherapy, targeted, immunotherapy or have acquired drug resistance, Chinese medicine compound with clear efficacy and non-toxicity has become a highly preferred choice. Therefore, the search for more effective antitumor drugs and molecules from TCM is of great research value.

Fufei Jiedu Prescription (FFJDF) is an empirical formula

created by Prof. Wang Yuanchun, who inherited the academic thought of "cancer elimination and detoxification" from Prof. Wu Mianhua, a "National Famous Traditional Chinese Medicine Practitioner", Which is composed of Astragalus, Radix et Rhizoma Ginseng, Ophiopogon, etc.. The whole formula achieves the effect of supporting the vital qi and dispelling the pathogens, anticancer and detoxification by regulating the yin and yang, complementing and chipping the treatment and using cold and warm herb together. Clinical application and controlled observation found that this formula in the treatment of NSCLC can control disease progression, improve quality of life, reduce clinical symptoms, stabilize or improve physical status scores, reduce NSCLC-sensitive tumor markers, improve hypercoagulability, and attenuate adverse effects of chemotherapy [7]. However, the specific molecular mechanism of this formula for the treatment of NSCLC is not clear, so in this study, we chose LUAD as the research object, constructed the interaction network of FFJDF-Target Pathway-LUAD by network pharmacology, and validated it by molecular docking technology to explore the mechanism of action of FFJDF in the treatment of LUAD.

2. Information and Methods

2.1 Prediction of the Active Ingredients and Potential Targets of the FFJDF

We utilize databases such as TCMSP, BATMAN, HERB and ETCM, the chemical constituents of each herbal medicine in FFJDF were collected, and oral bioavailability (OB) ≥30% and druglikeness (DL) ≥0.18 [8] were used as screening criteria to obtain the active ingredients of FFJDF. The Uniprot database (https://www.uniprot.org/) was then used to normalize the target names and eliminate overlapping terms to obtain the drug active ingredients and targets.

2.2 Screening the FFJDF and LUAD Related Targets

The author searched the DisGeNET, CTD and GeneCards databases for relevant targets using the keyword “lung adenocarcinoma”. and validated targets potentially related to LUAD were identified by aggregating and integrating these genes, normalizing them, and eliminating overlapping data. The intersections of FFJDF and LUAD targets screened in the previous step 1.1 were taken into R as potential targets for FFJDF treatment of LUAD for the next step of the analysis. Venn diagrams of target intersections between different databases were generated using the R4.2.1 VennDiagram1.7.3 and UpSetR1.4.0 packages.

2.3 FFJDF Component-Target LUAD Network Construction and Analysis

We implanted the actives and targets in FFJDF into Cytoscape 3.9.1 software for visualization and analysis, and the network diagram of herb-FFJDF Component-Target LUAD was constructed, and the structural characteristics of the network were analyzed using the Network Analyzer tool, which in turn calculated and ranked the connectivity degree value (degree) of each node.

2.4 Build and Analyze Protein-Protein Interaction Networks

The overlapping genes identified in section 1.2 were inserted into the STRING database to construct a protein-protein interaction (PPI) network diagram by us, selecting the species "Homo sapiens" and setting the confidence interval to ≥0.7 to obtain the protein-protein interaction relationships. The data was then imported into Cytoscape software in TSV file format to analyze the structure of the network, which was used to create a PPI network diagram. Finally, the data was ranked according to the degree of connectivity to obtain the key action targets.

2.5 GO Functional Enrichment and KEGG Pathway Enrichment Analysis

We performed gene ontology (GO) functional enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway-based enrichment analysis on the genes obtained in 1.2 using the R clusterProfiler 4.6.2 package. The cellular component (CC), biological process (BP), molecular function (MF) [9] and potential pathways of FFJDF acting on LUAD were screened with $P < 0.05$ as the criterion for significant difference.

2.6 Molecular Docking

We investigated the interactions between key compounds and core targets of FFJDF in the treatment of LUAD by molecular docking method. The 3D molecular structure information of the key active ingredients of FFJDF was extracted from the PubChem database, and the 3D structures of the key target proteins were downloaded from the Protein Data Bank (https://www.rcsb.org/) database. The Pymol 2.6.0 software was used for basic processing of the core target proteins, such as removal of solvent molecules. The AutoDock Tools 1.5.7 program was then used to add hydrogen atoms to these target proteins and assign appropriate charges. The processed target proteins and compounds were saved in "pdbqt" format with appropriate grid parameters. Molecular docking was performed by Autodock Vina and graphically displayed using PyMOL software.

3. Results and Analysis

3.1 Collection of FFJDF Active Ingredients and Target Prediction

We screened the active ingredients in FFJDF satisfying OB ≥ 30% and DL ≥ 0.18 through the drug database, and a total of 99 active ingredients were obtained after de-emphasis, among which 27 active ingredients in Astragalus membranaceus, 37 Hemerocallis sinensis, and 9 Tennessee and the main active ingredients are shown in Table 1. The database was used to obtain potential targets of action for 99 active ingredients, resulting in 956 drug targets, as shown in Figures 1 and 2.

Table 1: Major Active Components of FFJDF

Serial No.	Chemistry ID	Compound Name	OB	DL	Source Chinese Medicine
1	MOL000098	quercetin	46.43334812	0.27525	radix astragali、Hedyotis diffusa、Scutellaria barbata、Asparagi Radix
2	MOL000006	luteolin	36.16262934	0.24552	Pseudostellariae Radix、Scutellaria barbata
3	MOL000173	wogonin	30.68456706	0.22942	Scutellaria barbata
4	MOL002714	baicalein	33.51891869	0.20888	Scutellaria barbata
5	MOL000422	kaempferol	41.88224954	0.24066	radix astragali、Pseudostellariae Radix
6	MOL000417	Calycosin	47.75182783	0.24278	radix astragali
7	MOL002776	Baicalin	40.12360996	0.75264	Scutellaria barbata
8	MOL000358	beta-sitosterol	36.91390583	0.75123	Pseudostellariae Radix、Arisaema heterophyllum Blume、Hedyotis diffusa、Scutellaria barbata、Bolbostemmatis Rhizoma、radix astragali、Asparagi Radix
9	MOL010861	Delta-D	45.65937895	0.47657	Ophiopogon japonicus、Hedyotis diffusa
10	MOL000354	isorhamnetin	49.60437705	0.306	radix astragali
11	MOL001689	acacetin	34.97357273	0.24082	Pseudostellariae Radix
12	MOL002773	beta-carotene	37.18433337	0.58358	radix astragali
13	MOL000392	formononetin	69.67388061	0.21202	radix astragali
14	MOL004328	naringenin	59.29389773	0.21128	Scutellaria barbata
15	MOL000546	diosgenin	80.87792491	0.80979	Asparagi Radix、Ophiopogon japonicus

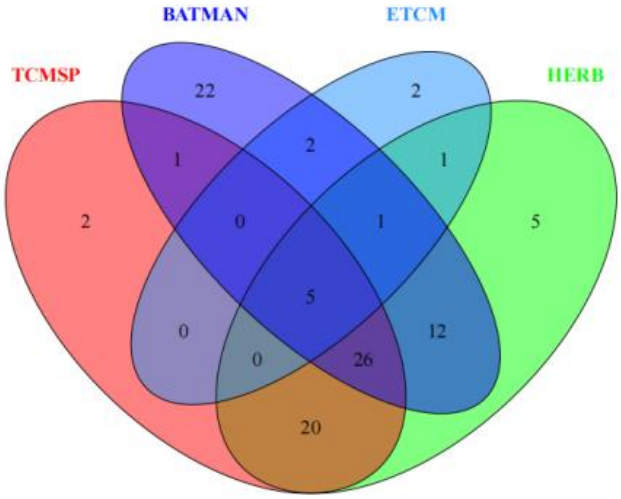

Figure 1: Intersection of active ingredients between different databases

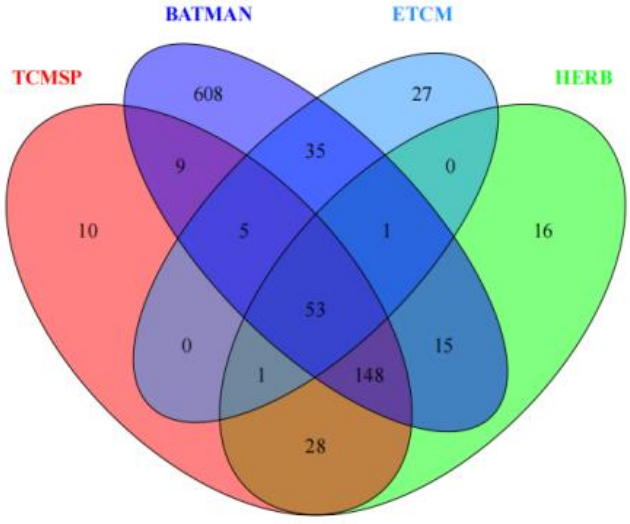

Figure 2: Intersection map of targets between different databases

3.2 Screening of FFJDF and LUAD-related Targets

We obtained a total of 582 LUAD-related targets through the DisGeNET database. After the intersection with the effective component target of FFJDF, 121 common targets were determined, and the Venn diagram was drawn (Figure 3).

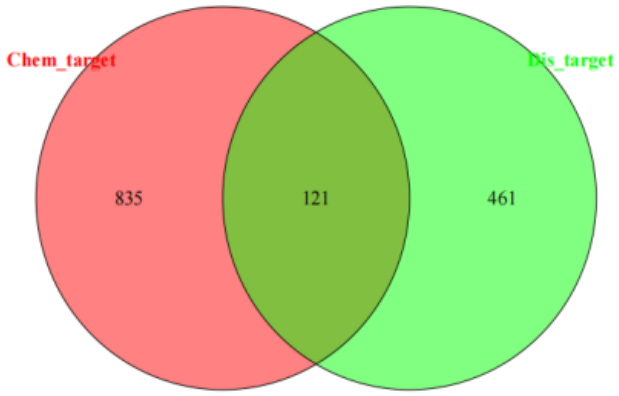

Figure 3: Intersection map of FFJDF and LUAD key targets

3.3 Construction and Analysis of FFJDF-component-target-LUAD Network

The network diagram of 'FFJDF-component-target-LUAD' was constructed by Cytoscape 3.9.0 software (Figure 4). There were 215 nodes and 867 edges, of which 82 intersectional active components acted on 121 targets. We use the Network Analyzer plug-in analysis to reveal the central nodes in the network. The number of edge connections (degree) of the nodes shows their importance in the network, and the top 20 active ingredients are plotted in a histogram (Figure 5). According to the degree > 30, five important active ingredients such as quercetin, luteolin, wogonin, baicalein and kaempferol were screened out. These compounds interact closely with multiple targets.

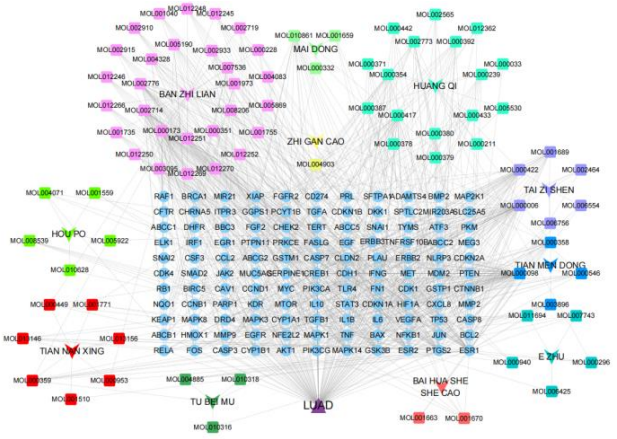

Figure 4: FFJDF-compound-target-disease network diagram

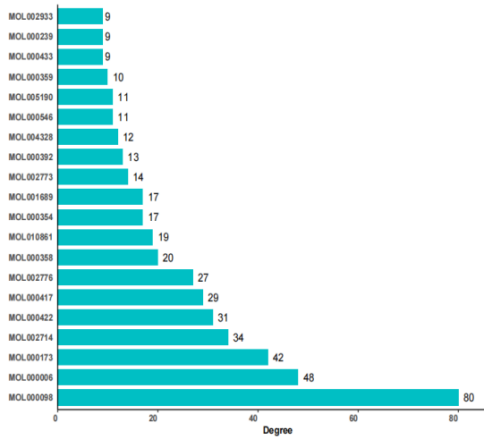

Figure 5: Histogram of the ordering of the connectivity of the compounds

3.4 PPI Network Diagram Analysis

We obtained a drug-disease common target interaction map of 111 nodes and 1288 edges through STRING database and Cytoscape program (Figure 6). The PPI interaction network was drawn by the Network Analyzer plug-in (Figure 7). The darker the color, the higher the connection strength. According to the degree > 30, 30 core proteins were screened and the histogram was drawn (Figure 8). The top 7 target proteins with network connectivity were TP53, AKT1, STAT3, EGFR, MYC, BCL2 and CTNNB1.

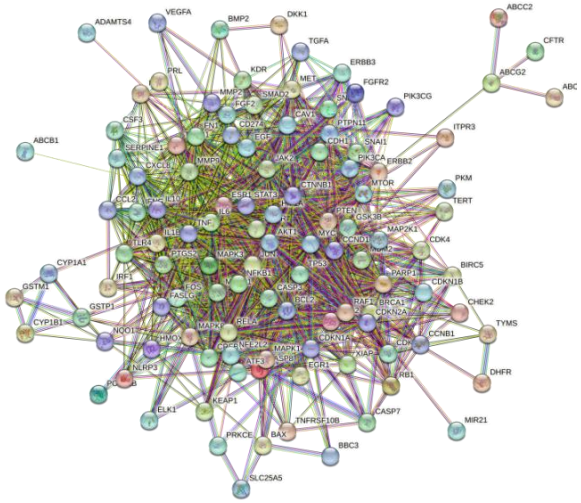

Figure 6: Drug-disease co-target interactions

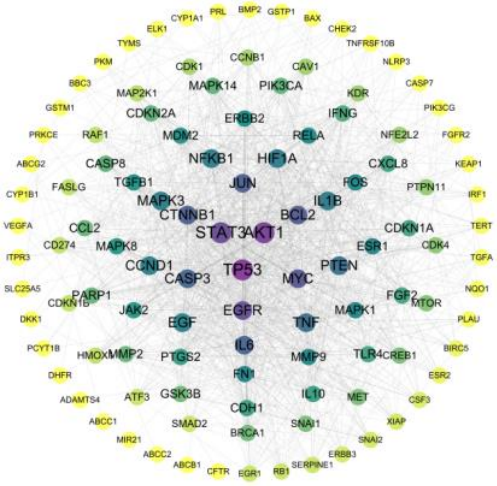

Figure 7: PPI interactions network diagram

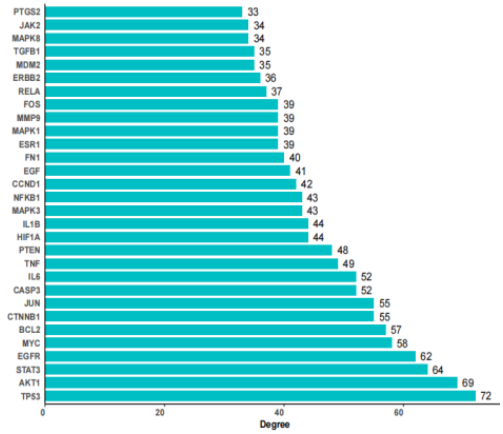

Figure 8: Histogram of core target connectivity ordering

3.5 GO Functional Enrichment Analysis and KEGG Pathway Analysis

We analyzed the abundance of GO functions of 121 common targets. According to P < 0.05, 1864, 178 and 88 were screened out for BP, MF and CC, respectively. The first 15 items with the smallest P values in each category were selected and displayed in the form of histogram (Figure 10). The cellular components of FJDF are mainly located in cell membrane rafts, cytoplasmic vesicle cavities, membrane microthresholds, PML bodies, transcriptional regulatory proteins or inhibitors, and complexes. The biological processes involved are signal transduction, apoptosis process, immune regulation, oxidative stress, biological regulation and so on. Its molecular function is mainly related to protease activity, protein binding, receptor and ligand binding, The binding of transcriptional regulatory factors is related to the activity of signal receptors. KEGG pathway enrichment analysis was performed on the target, and a total of 157 signaling pathways were found. According to P < 0.05, 44 pathways were screened out, and the first 30 pathways with significant differences were visualized by bubble diagram (Figure 11). The results showed that the signaling pathways mainly involved in Phosphatidylinositol 3 kinase (PI3K) -protein kinase B (Akt), AGE-RAGEwhich is in the diabetic complications (AGE-RAGE in diabetic complications), umor necrosis factor (TNF) and programmed cell death-ligand 1 (PD-1) checkpoints etc.

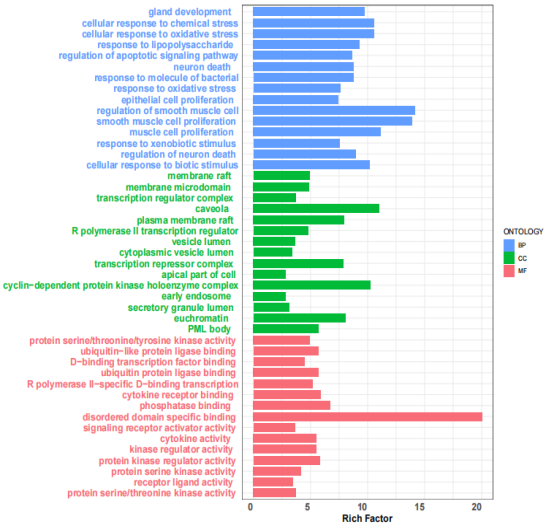

Figure 9: Histogram of GO enrichment analysis of potential targets

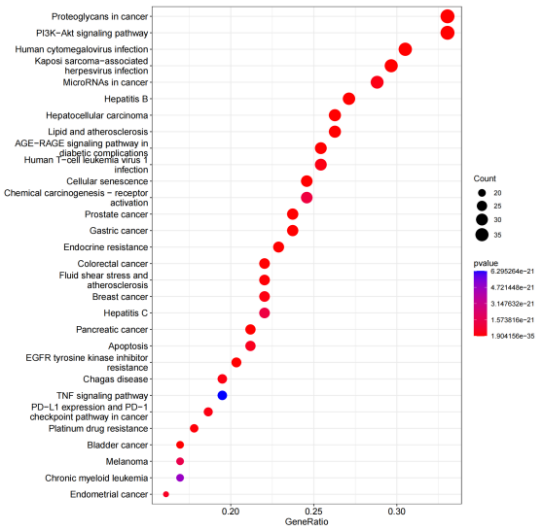

Figure 10: Bubble plot of KEGG enrichment analysis of potential pathways

3.6 Molecular Docking

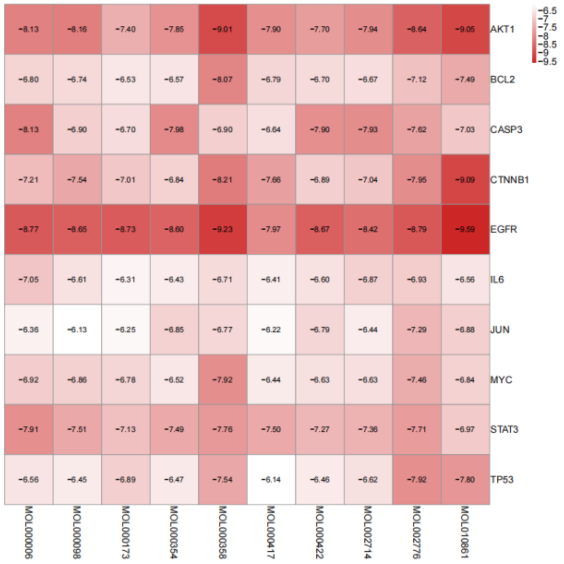

Figure 11: Docking energy thermogram of Top active ingredients and Top targets

We performed molecular docking of the top 10 compounds with the important target proteins obtained in PPI, and the molecular docking is shown in Figure 11. The results showed that the main compounds were well docked with the key targets, and the binding energy was < -6.5 kcal / mol. Specifically, the binding energy of AKT1 and EGFR proteins to all active ingredients was < -7.0 kcal / mol; the binding energies of AKT1 and EGFR with all active ingredients were < -7.0 kcal / mol; The binding energy of STAT3 with quercetin, luteolin, wogonin, baicalein, kaempferol, calycosin, baicalin, β-sitosterol and isorhamnetin was < -7.0 kcal / mol. The binding energy of CTNNB1 with quercetin, luteolin, wogonin, baicalein, calycosin, baicalin, β-sitosterol and Delta-D was < -7.0 kcal / mol. The binding energy of CASP3 with luteolin, isorhamnetin, Delta-D, baicalin, baicalein and kaempferol was < -7.0 kcal / mol. The binding energy of BCL2 with baicalin, β-sitosterol and Delta-D was < -7.0 kcal / mol. The binding energy of TP53 with isorhamnetin, baicalin and Delta-D was < -7.0 kcal / mol. The binding energy of MYC with isorhamnetin and baicalin was < -7.0 kcal / mol. The binding energies of IL6 and luteolin, JUN and baicalin were all < -7.0 kcal / mol. The binding energies of EGFR, AKT1 with β-sitosterol and Delta-D, CTNNB with Delta-D were all < -9.0 kcal / mol. The docking structures of the top 6 targets and compounds were simulated by PyMOL. The simulation showed that AKT1, EGFR, CTNNB1 and Delta-D, EGFR and baicalin can form two or more hydrogen bonds, AKT1 and EGFR and β-sitosterol form a hydrogen bond. The docking situation is shown in Figure 14.

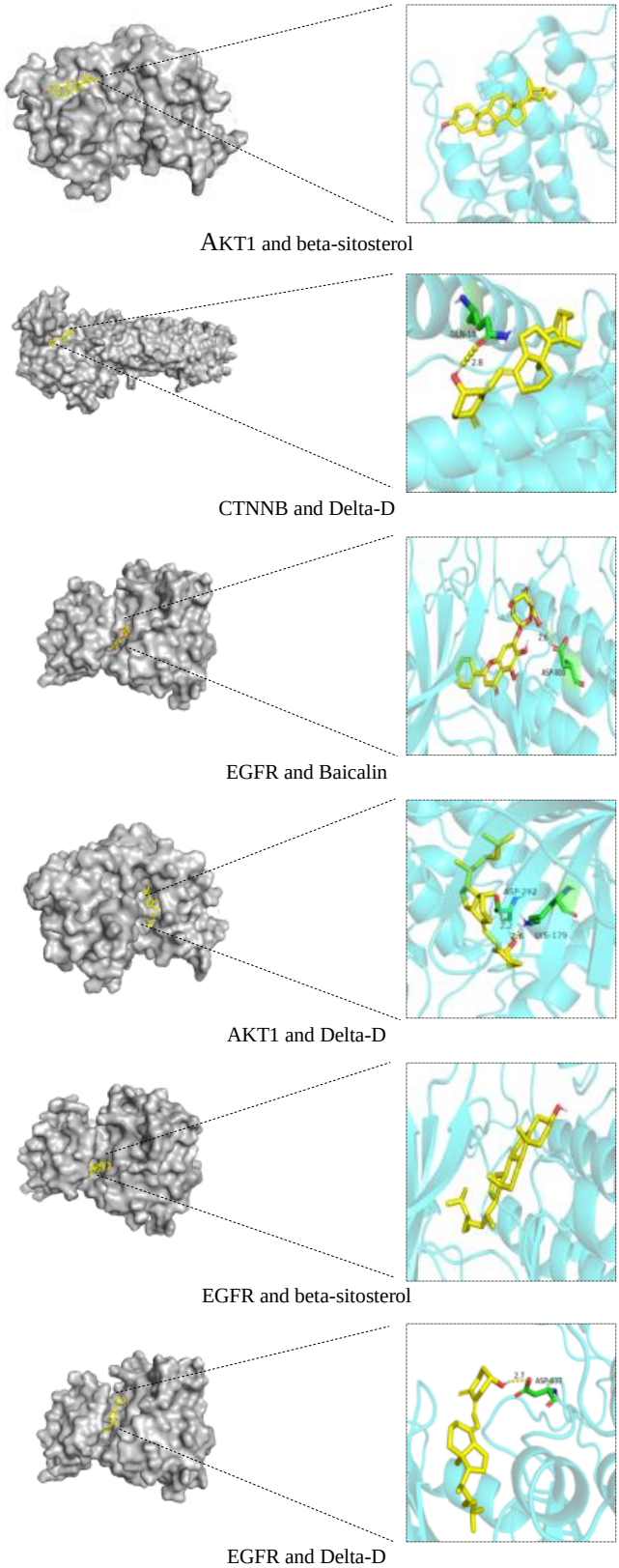

Figure 12: Detail of molecular docking of AKT1, CTNNB and EGFR

4. Discussions

Although there are many treatment methods for LUAD, in addition to surgery, radiotherapy and chemotherapy, molecular targeted and immunotherapy drugs are widely used in clinical practice, but the efficacy gain is minimal and the prognosis is poor. A number of studies have shown that traditional Chinese medicine has significant advantages in improving clinical symptoms, improving clinical efficacy, increasing the rate of 'survival with tumor', and reducing adverse reactions caused by various chemical drugs [9-11]. FFJDF is an empirical formula based on the pathogenesis theory of 'cancer toxin'. Radix astragali and prince ginseng in the prescription tonify the qi of lung -spleen; Cochinchinese Asparagus Root and Ophiopogon japonicus benefit the yin of lung-stomach, nourish kidney-water, and supplement the yin of the whole body. Bolbostemmatis Rhizoma dissipating phlegm to remove mass; Hedyotis diffusa、Scutellaria barbata Detoxification and elimination cancer; Acruginous Turmeric Rhizome Untie the blood stasis and elimination product; Officinal magnolia bark eliminating accumulation and relieving asthma, dredging fu-organ qi to reduce the qi of lung; Liquorice root Reconciliation of various drugs and anti-poisonous. The whole formula is based on 'difficiency of vital qi', 'Cancer toxin' as fundamental, created by the physiological characteristics of the lung viscera, Jointly exert the effects of nourishing qi and yin, resolving phlegm and blood stasis, detoxifying and eliminating cancer. Further metabolomics studies have found that FFJDF can regulate the synthesis and metabolism of compounds such as alcohols, steroids, glycerides, organic acids, and acylcarnitines in the body, affecting the expression of genes and functional proteins, thereby altering the tumor microenvironment, promoting immune response, and controlling the progression of NSCLC [7]. However, the specific molecular mechanism of FFJDF in the treatment of LUAD is still unclear. Exploring this mechanism is of great significance for further improving the clinical efficacy of LUAD and promoting the research and development of FFJDF.

In this study, 121 genes related to FFJDF and LUAD were found by network pharmacology. Quercetin, luteolin, wogonin, baicalein and kaempferol were the main components of therapeutic effect. PPI analysis revealed that FFJDF targeted core genes such as TP53, AKT1, STAT3, EGFR, MYC, BCL2 and CTNNB1 to treat LUAD. In Asian NSCLC patients, about 50 % of patients have epidermal growth factor receptor (EGFR) mutations [12]. TP53, PI3K, CTNNB1 and other genes are often co-mutated with EGFR to increase the malignancy of LUAD [13]. EGFR mutation leads to the activation of tyrosine kinase in the cytoplasm. Tyrosine phosphorylation binds to downstream signaling pathways such as P13K / Akt, JAK / STAT and RAS / RAF / MAPK, stimulates cell growth, affects cell proliferation and differentiation, and leads to tumorigenesis and even metastasis [14]. A study of 110 cases of NSCLC tumors showed that 51 % of Akt activity was increased by immunohistochemistry, and AKT1 was a key target of PI3K / Akt pathway [15]. Activated AKT inhibits the pro-apoptotic ability of Bcl2 protein family members, down-regulates the transcription of forkhead transcription factors of the O class (FOXO) and activated B cells (NFkB), promotes the activation of mammalian target of rapamycin (mTOR), leads to increased cell survival and anti-apoptotic signal expression [16], and affects the metabolic reprogramming of tumor cells. Eventually lead to the occurrence of lung cancer [17]. CTNNB1 is an intracellular scaffold protein. When the expression is active, the CTNNB1 protein accumulated in the cytoplasm penetrates into the nucleus and binds to the transcriptional activator group TCF / LEF [18-19], triggering the initiation of the Wnt pathway and affecting cell growth activity. Available evidence suggests that activation of the Wnt pathway is common in human NSCLC [20-21] Overexpression of BCL2 inhibits programmed cell death and apoptosis induced by external stimuli (such as DNA damage or anticancer drugs including platinum drugs) [22-23]. A number of studies have found that the up-regulation of TP53, STAT3 and MYC is often related to the poor prognosis and therapeutic drug resistance of LUAD [24-26]. Through the above analysis, the main active components in FFJDF may improve the clinical symptoms and prolong the survival time of patients by affecting the proliferation, activation, apoptosis, immunity and metabolism of LUAD cells.

GO functional enrichment analysis showed that cell signal transduction, apoptosis, immune regulation, oxidative stress and biological regulation were the main biological processes of FFJDF intervention in LUAD, and the dynamic correlation between the biological processes affected the occurrence and development of LUADThe results of KEGG enrichment showed that the signaling pathways were mainly involved in PI3K-Akt, AGE-RAGE, TNF and PD-1 checkpoint signaling pathways etc. Studies have confirmed that the PI3K / Akt pathway in NSCLC regulates a variety of cellular processes, such as survival, proliferation, migration, angiogenesis, cell metabolism, cell senescence, genomic integrity and stem cell self-renewal [27-28]. Studies have shown that inhibition of some major components of the PI3K pathway can inhibit cancer growth [29]. The combination of baicalein and ametinib can improve the anti-tumor activity of ametinib-resistant NSCLC through the ROS-mediated PI3K-Akt pathway [30]. Some scholars have found that baicalin and β-sitosterol can inhibit the invasion and metastasis of NSCLC cells by inhibiting the activation of PI3K-AKT pathway, inducing Akt-dependent cell cycle arrest, promoting apoptosis, reversing EMT expression, and interfering with the immune microenvironment [31-35]. In adults, RAGE has the highest expression level in lung tissue [36], but RAGE expression is significantly down-regulated in NSCLC [37-38]. Studies have found that overexpression of RAGE in lung cancer cells inhibits the proliferation of lung cancer cells [39]. However, it is contradictory that although RAGE is reduced in lung cancer patients, its ligand AGES is increased [40-41]. AGES is a late glycosylation end product, and cancer cells have high aerobic glycolysis and metabolic capacity. The enrichment of AGES promotes the proliferation effect and oxidative stress response of cancer cells [42]. Therefore, the AGE / RAGE signaling pathway may contribute to the tumorigenic process of lung cancer by promoting chronic inflammation, regulating the tumor microenvironment, and affecting cell survival pathways [43]. TNF signaling pathway involves multiple cellular processes, including cell survival, proliferation, angiogenesis and death [44]. A control study showed that the expression of TNF-α mRNA in NSCLC tumor tissues was significantly higher than that in adjacent normal tissues [45]. Tumor necrosis factor

(TNF-α) is an important regulator of tumor microenvironment, which can be used as a promoter to directly lead to inflammation and promote tumor formation [46]. In addition, TNF activation can bind to its receptors TNFR1 and TNFR2, and induce multiple downstream pathways including NF-kappa B, JNK, MAPK to regulate the immune response, proliferation, apoptosis, invasion and migration of lung cancer cells [47-49]. Chang et al. found that polysaccharides extracted from Astragalus membranaceus and Codonopsis pilosula can up-regulate the secretion of IL-6 and TNF-α to achieve anti-cancer effects [50]. PD-L1 is highly expressed in NSCLC [51]. Binding with PD-1 leads to a decrease in the immune effect of T cells in the tumor microenvironment, which mediates tumor immune escape and promotes tumor growth. It has been reported that baicalein can down-regulate PD-L1 expression by inhibiting STAT3 activity, and then re-activate T cell sensitivity to kill tumor cells [52]. Therefore, FFJDF may play an anti-tumor role by regulating PI3K / Akt, AGE / RAGE, TNF, PDL-1 and other signaling pathways.

According to the results of molecular docking, the top ten active compounds including quercetin, luteolin, wogonin, baicalein, kaempferol, calycosin, baicalin, β-sitosterol, Delta-D and isorhamnetin had a binding capacity of < -6.5 kcal / mol to key proteins (including TP53, AKT1, STAT3, EGFR, MYC, BCL2, CTNNB1, JUN, CASP3 and IL6), indicating that they had high affinity with proteins. It is generally believed that only when the binding energy between the substance and the protein is lower than 0 kcal / mol, the two can spontaneously bind; the binding energy < -5 kcal / mol indicates that the affinity is good, and the binding energy < -7.5 kcal / mol indicates that the stability of the molecular interaction is extremely strong [39]. Among them, the binding energies of AKT1, CTNNB1 and EGFR with quercetin, calycosin, baicalin, β-sitosterol, Delta-D and other main components were all < -9.0kcal / mol, and the binding was very tight, which indicated that quercetin, calycosin, baicalin, β-sitosterol and Delta-D, AKT1, CTNNB1 and EGFR were likely to be the effective components and binding targets of FFJDF.

In summary, FFJDF may act on EGFR, AKT, CTNNB1 and other targets through various components such as quercetin, calycosin, baicalin, β-sitosterol and Delta-D, regulate PI3K-AKT, PI3K / Akt, AGE / RAGE, TNF and PDL-1 signaling pathways, inhibit tumor activity, promote apoptosis, block angiogenesis, regulate immune microenvironment, and improve the bioavailability of synergistic drugs to exert anti-tumor effects. This study preliminarily predicted the synergistic mechanism of multi-target, multi-component and multi-pathway of FFJDF intervention in LUAD, but only the target genes of LUAD in DisGeNET, CTD and GeneCards databases were screened, and the data were incomplete. It may lead to the omission of the target and affect the comprehensiveness and accuracy of the results. In the future, quantitative analysis of FFJDF components and effective components into the blood can be further carried out, and then in vitro and in vivo experiments can be gradually carried out from the molecular mechanism to study the specific utility mechanism of FFJDF from multiple perspectives and levels.

Funding Project

Xianyang Science and Technology Bureau project (2020K02-95)

References

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May-Jun;74(3):229-263.

[2] heng RS, Chen R, Han BF, et al. [Cancer incidence and mortality in China, 2022]. Zhonghua Zhong Liu Za Zhi. 2024 Mar 23;46(3):221-231.

[3] H. M. Abdelaziz, M. Gaber, M. M. Abd-Elwakil, et al. Elzoghby Inhalable particulate drug delivery systems for lung cancer therapy: Nanoparticles, microparticles, nanocomposites and nanoaggregates J. Control Release, 269 (2018), pp. 374-392

[4] Roberto Ruiz-Cordero, Walter Patrick Devine, Targeted Therapy and Checkpoint Immunotherapy in Lung Cancer, Surgical Pathology Clinics, Volume 13, Issue 1, 2020, Pages 17-33

[5] Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res. 2019 Sep 15; 79(18): 4557-4566.

[6] Gettinger S, Horn L, Jackman D, et al. Five-year follow-up of nivolumab in previously treated advanced non-small-cell lung cancer: results from the CA209-003 study [J]. J Clin Oncol, 2018, 36(17): 1675-1684.

[7] Wang Yuanchun. Study of Clinical and Metabolomics on the Treatment of Non-small Cell Lung Cancer Based on Pathogenesis Theory of Cancerous toxin[D]. Nanjing University of Chinese Medicine, 2022.

[8] Wang Xiaoyu. Meta-analysis and Network Pharmacology Study of Guipi Decoction in the treatment of Post-Stroke Depression[D]. Shaanxi University of Chinese Medicine, 2022.

[9] ZHANG Xiaowen, LIU Aimin, ZHAO Jingjing, et al. Mechanism of Mahuang Lianqiao Chixiaodou Decoction in treating eczema by network pharmacology and molecular docking technology[J]. China Journal of Chinese Materia Medica,2021,46(04):894-901.

[10] DENG Zhengting, ZHAO Fan, ZHAO Tong, et al. Analysis on Medication Regularity of Traditional Chinese Medicine in Treatment of Intermediate and Advanced Non-small Cell Lung Cancer Based on Data Mining. [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2022,28(3):171-179.

[11] ZHOU Mei, KOU Yan, WANG Hongli. Effect of Modified Shengxian Decoction Combined with TP Chemotherapy on Non-Small Cell Lung Cancer and Its Influence on Inflammatory Cytokines and Immune Function. [J]. Liaoning Journal of Traditional Chinese Medicine, 2022,49(6): 84-87.

[12] Lu J, Li J, Lin Z,et al. Reprogramming of TAMs via the STAT3/CD47-SIRPα axis promotes acquired resistance to EGFR-TKIs in lung cancer. Cancer Lett. 2023 Jun 28;564:216205.

[13] Blakely CM, Watkins TBK, Wu W, et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat Genet al. 2017 Dec;49(12):1693-1704.

[14] Zheng Q, Dong H, Mo J, et al. A novel STAT3 inhibitor W2014-S regresses human non-small cell lung cancer xenografts and sensitizes EGFR-TKI acquired resistance. Theranostics. 2021 Jan 1;11(2):824-840.
[15] Balsara BR, Pei J, Mitsuuchi Y, et al. Frequent activation of AKT in non-small cell lung carcinomas and preneoplastic bronchial lesions. Carcinogenesis 2004; 25: 2053–9.
[16] Tan AC. Targeting the PI3K/Akt/mTOR pathway in non-small cell lung cancer (NSCLC). Thorac Cancer. 2020 Mar;11(3):511-518.
[17] Moes-Sosnowska J, Szpechcinski A, Chorostowska - Wynimko J. Clinical significance of TP53 alterations in advanced NSCLC patients treated with EGFR, ALK and ROS1 tyrosine kinase inhibitors: An update. Tumour Biol. 2024;46(s1):S309-S325.
[18] E. D. Kramer, S. L. et al. Abrams β-Catenin signaling in alveolar macrophages enhances lung metastasis through a TNF-dependent mechanism JCI Insight, 8 (8) (2023), 10. 1172.
[19] Katoh M. Multi-layered prevention and treatment of chronic inflammation, organ fibrosis and cancer associated with canonical WNT/β-catenin signaling activation (Review). Int J Mol Med. 2018 Aug; 42(2): 713-725.
[20] Y Liao, J Feng, W Sun, et al. CIRP promotes the progression of non-small cell lung cancer through activation of Wnt/β-catenin signaling via CTNNB1. J. Exp. Clin. Cancer Res., 40 (1) (2021), p. 275
[21] Green DR. Apoptotic Pathways: Ten Minutes to Dead. Cell. 2005; 121: 671–674.
[22] Lindner AU, Concannon CG, Boukes GJ, et al. Systems analysis of BCL2 protein family interactions establishes a model to predict responses to chemotherapy. Cancer Res. 2013; 73: 519–528.
[23] Liu X, Shao Y, Zhang X, et al. Calycosin attenuates pulmonary fibrosis by the epithelial-mesenchymal transition repression upon inhibiting the AKT/GSK3β/β-catenin signaling pathway. Acta Histochem. 2021 Jul;123(5):151746.
[24] Carpten JD, Faber AL, Horn C, et al. A Transforming Mutation in the Pleckstrin Homology Domain of AKT1 in Cancer. Nature. 2007; 448: 439–444.
[25] Landgraf KE, Pilling C, Falke JJ, et al. Molecular Mechanism of an Oncogenic Mutation That Alters Membrane Targeting: Glu17Lys Modifies the PIP Lipid Specificity of the AKT1 PH Domain. Biochemistry. 2008; 47: 12260–12269.
[26] Le X, Nilsson M, Goldman J, et al. Dual EGFR-VEGF Pathway Inhibition: A Promising Strategy for Patients With EGFR-Mutant NSCLC. J Thorac Oncol. 2021 Feb;16(2):205-215.
[27] Losuwannarak N, Maiuthed A, Kitkumthorn N, et al. Gigantol targets cancer stem cells and destabilizes tumors via the suppression of the PI3K/AKT and JAK/STAT pathways in ectopic lung cancer xenografts. Cancers. 2019; 11: 2032.
[28] Liu F, Gao S, Yang Y, et al. Antitumor activity of curcumin by modulation of apoptosis and autophagy in human lung cancer A549 cells through inhibiting PI3K/Akt/mTOR pathway. Oncol. Rep. 2018; 39: 1523–1531.
[29] Dimri M, Humphries A, Laknaur A, et al. NAD (P) H quinone dehydrogenase 1 ablation inhibits activation of the phosphoinositide 3-kinase/Akt serine/threonine kinase and mitogen-activated protein kinase / extracellular signal-regulated kinase pathways and blocks metabolic adaptation in hepatocellular carcinoma. Hepatology. 2020; 71: 549–568.
[30] Chen T, Zhang P, Cong XF, et al. Synergistic antitumor activity of baicalein combined with lmonertinib in almonertinib-resistant non-small cell lung cancer cells through the reactive oxygen species-mediated PI3K/Akt pathway. Front Pharmacol. 2024 Jul 31;15:1405521.
[31] Lindner AU, Concannon CG, Boukes GJ, et al. Systems analysis of BCL2 protein family interactions establishes a model to predict responses to chemotherapy. Cancer Res. 2013;73:519–528.
[32] E. D. Kramer, S. L. et al. Abrams β-Catenin signaling in alveolar macrophages enhances lung metastasis through a TNF-dependent mechanism JCI Insight, 8 (8) (2023), 10. 1172.
[33] Katoh M. Multi-layered prevention and treatment of chronic inflammation, organ fibrosis and cancer associated with canonical WNT/β-catenin signaling activation (Review). Int J Mol Med. 2018 Aug;42(2):713-725.
[34] GUO Xiaofei, ZHANG Ping, BAI Jianqi, et al. Molecular Mechanism of Guben Jiedu Prescription in Regulating Immune Microenvironment of Lung Adenocarcinoma and Experimental Verification[J]. World Chinese Medicine, 2023, 18 (20): 2865-2871+2877.
[35] LIU Huanyu, WANG Yanbing, YANG Tao, et al. Potential Action Mechanism of Qijia Fuzheng Formula in the Treatment of Cancer Related Fatigue of Lung Adenocarcinoma Based on Network Pharmacology and Molecular Docking [J]. World Chinese Medicine, 2021, 16 (11): 1685-1691.
[36] Schraml P, Bendik I, Ludwig CU. Differential messenger RNA and protein expression of the receptor for advanced glycosylated end products in normal lung and non-small cell lung carcinoma. Cancer Res. 1997;57(Sep (17)):3669–71.
[37] Stav D, Bar I, Sandbank J. Usefulness of CDK5RAP3, CCNB2, and RAGE genes for the diagnosis of lung adenocarcinoma. Int J Biol Markers. 2007;22(Apr–Jun (2)):108–13. doi: 10. 1177/172460080702200204.
[38] BI. Alternatively spliced RAGEv1 inhibits tumorigenesis through suppression of JNK signaling. Cancer Res. 2010;70(Jul (13)):5628–38.
[39] Wang H, Li Y, Yu W, et al. Expression of the receptor for advanced glycation end-products and frequency of polymorphism in lung cancer. Oncology letters. 2015;10(Jul (1)):51–60.
[40] Beer DG, Kardia SL, Huang CC, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med. 2002;8(Aug (8)):816–24.
[41] Diederichs S, Bulk E, Steffen B, et al. S100 family members and trypsinogens are predictors of distant metastasis and survival in early-stage non-small cell lung cancer. Cancer Res. 2004;64(Aug (16)):5564–9.
[42] Rojas A, Figueroa H, Morales E. Fueling inflammation at tumor microenvironment: the role of

multiligand/RAGE axis. Carcinogenesis. 2010;31(Mar (3)):334–41.
[43] Oczypok EA, Perkins TN, Oury TD. All the "RAGE" in lung disease: The receptor for advanced glycation endproducts (RAGE) is a major mediator of pulmonary inflammatory responses. Paediatr Respir Rev. 2017 Jun; 23:40-49.
[44] Bhat IA, Mir IR, Malik GH, et al. Comparative study of TNF-α and vitamin D reveals a significant role of TNF-α in NSCLC in an ethnically conserved vitamin D deficient population. Cytokine. 2022 Dec;160:156039.
[45] Huyghe J, Priem D, Bertrand MJM. Cell death checkpoints in the TNF pathway. Trends Immunol. 2023 Aug;44(8):628-643.
[46] ZHU Lingling, ZHANG Yani, SHI Tingting, et al. The role of tumor necrosis factor-α in the development of hepatocellular carcinoma[J]. Journal of Clinical Hepatology,2024,40(11):2320-2325.
[47] SHI Huimin, SU Jie, LIU Huijin. Effects of TNF-αon the Biological Behavior of Lung Cancer Cells by Regulating NF-κB/PXR Inflammatory Pathway and Its Mechanism [J]. The Practical Journal of Cancer, 2024, 39(08): 1219-1223.
[48] 79Wang C, Wen M, Xu J, et al. GTSE1 promotes the growth of NSCLC by regulating microtubule-associated proteins through the ERK/MAPK pathway. Thorac Cancer. 2023 Jun;14(17):1624-1634. doi: 10. 1111/1759-7714. 14908. Epub 2023 Apr 20.
[49] Xiong Ying, Shen Jingqiao, Liu Xianguo, et al. Effects of magnesium -L- threonate on migration and invasion ability and NF-κB/TNF-alpha signaling pathway of non -small cell lung cancer H460 cells[J]. Journal of Modern Oncology, 2024, 32(24): 4580-4587.
[50] Chang W. T, Lai TH, Chyan YJ, et al. Specific medicinal plant polysaccharides effectively enhance the potency of a DC-based vaccine against mouse mammary tumor metastasis. PloS One. 2015;10
[51] Farrag M, Ibrahim E, Abdelwahab H, et al. PDL-1 expression in lung carcinoma and its correlation with clinicopathological and prognostic characteristics. J Immunoassay Immunochem. 2021 Nov 2; 42(6): 679-690.
[52] Ke M, Zhang Z, Xu B, et al. Baicalein and baicalin promote antitumor immunity by suppressing PD-L1 expression in hepatocellular carcinoma cells. Int Immunopharmacol. 2019;75:105824.