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Research article

Combinational study with network pharmacology, molecular docking and preliminary experiments on exploring common mechanisms underlying the effects of weijing decoction on various pulmonary diseases

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ABSTRACT

Objective: 'Homotherapy for heteropathy' is a theory by which different diseases with similar pathogenesis can be treated with one Chinese formula. We aimed to explore the key components and core targets of Weijing decoction (WJD) in treating various lung diseases, namely, pneumonia, chronic obstructive pulmonary disease (COPD), acute lung injury (ALI), pulmonary fibrosis, pulmonary tuberculosis and non-small cell lung cancer (NSCLC), via network pharmacology, molecular docking and some experiments.

Significance: This is the first study on the mechanism of WJD in treating various lung diseases by 'homotherapy for heteropathy'. This study is helpful for the transformation of TCM formula and development of new drugs.

Methods: Active components and therapeutic targets of WJD were obtained via TCMSP and UniProt databases. Targets of the six pulmonary diseases were harvested from the GeneCards TTD, DisGeNet, UniProt and OMIM databases. Drug-disease intersection targets, corresponding Venn diagrams, herb-component-target networks and protein–protein interaction networks were established. Furthermore, GO biological function and KEGG enrichment analysis were completed. Moreover, the binding activity between main compounds and core targets was measured through molecular docking. Finally, the xenograft NSCLC mouse model was established. Immune

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responses were evaluated by flow cytometry and mRNA expression levels of critical targets were measured by real-time PCR.
Results: JUN, CASP3 and PTGS2 were the most critical targets in six pulmonary diseases. The active compounds beta-sitosterol, tricin and stigmasterol stably bound to many active sites on target proteins. WJD had extensive pharmacological regulation, involving pathways related to cancer, inflammation, infection, hypoxia, immunity and so on.
Conclusions: Effects of WJD against various lung diseases involve lots of compounds, targets and pathways. These findings will facilitate further research as well as clinical application of WJD.

1. Introduction

Studies have shown that 10,000 L of air is inhaled into the lungs every 24 h. Thus, we are exposed to a large number of suspended particles, toxic gases, air allergens and microorganisms which can possibly cause pulmonary diseases. Although the protective mechanism of the lungs is effective in most days, the number of people hospitalized due to lung diseases has increased because pollution is a serious problem, and COVID-19 is becoming increasingly prevalent [1]. Pulmonary diseases mainly include pneumonia, chronic obstructive pulmonary disease (COPD), acute lung injury (ALI), pulmonary fibrosis, pulmonary tuberculosis and lung cancer such as non-small cell lung cancer (NSCLC). These six types of diseases have similar pathogenesis such as inflammatory reaction, immune response and oxidative stress, which can be treated through similar therapies [2–5]. This feature is consistent with the theory of 'homotherapy for heteropathy' in traditional Chinese medicine (TCM).

When fighting the COVID-19 pandemic in Wuhan, the Chinese government recommended several Chinese formulas including *Lianhua Qingwen* capsules, *Qing-Fei-Pai-Du* decoction, *Hua-Shi Bai-Du* decoction as well as *Xuan-Fei-Pai-Du* decoction for infected patients. These Chinese prescriptions achieve satisfactory results, so people have growing confidence in using Chinese medicines to treat such diseases [6]. In clinical settings, some classical Chinese formulas are usually used to treat pulmonary diseases. For example, Jiawei-Yupingfeng-Tang is used in the treatment of pneumonia [7]; Xiaoqinglong decoction is widely used in COPD [8]; Liang-Ge-San is used to treat ALI [9]; BuqiHuoxueTongluo Formula plays an important role in pulmonary fibrosis [10]; Ma-Xing-Gan-Shi-Tang has a crucial effect on pulmonary tuberculosis [11]; and Ze-Qi-Tang is used for the treatment of NSCLC [12]. Various TCM compounds can be used for the treatment of lung diseases, and some prescriptions can even be used to treat a variety of different diseases. Weijing decoction (WJD) contains four herbs such as *Rhizoma Phragmitis, Coicis Semen, Persicae Semen* and *Benincasae Semen.* Not only can it be used to treat COPD, but it can also be used for the treatment of NSCLC, COVID-19 and other pulmonary diseases [13–15]. In China, WJD is used in clinical research on common respiratory diseases such as pneumonia, COPD, lung cancer and pulmonary abscess. Reports related to hospital-acquired pneumonia showed that, combining with WJD and symptomatic treatment of Western medicine, the total effective rate was significantly improved (83.0% in the research group and 64% in the control group), and the occurrence of adverse reactions was improved. Liu Yan et al. treated 40 patients with AECOPD with the combination of WJD and Maxing Shigan Decoction (MXSGD) combined with conventional Western medicine therapy, which could better relieve the symptoms of dyspnea, cough and sputum, and reduce the serum TNF-α level. Liu Donghu et al., on the basis of chemotherapy combined with paclitaxel and cisplatin, used WJD and Qingre Paifu Decoction to treat 46 patients with lung cancer, and the tumor control rate was significantly improved (84.78% in the study group and 65.22% in the control group). What's more, Liang Jiajia et al. used modified WJD combined with bronchoscopic alveolar lavage to treat 37 patients with acute lung abscess, which could effectively shorten the time for symptoms to disappear. Clinical trial ChiCTR2000030759 showed that WJD combined MXSGD improved recovery rate of symptoms in COVID-19 pandemic. However, the potentially common mechanisms of WJD in treating lung diseases should be elucidated.

In recent years, network pharmacology has been frequently used in the research of TCM prescriptions, and it is suitable for research on prescriptions. Through searching for numerous databases, bioactive compound screening, target discovery and pathway enrichment can be completed. The herb-compound-target-pathway network can be constructed to realize the transformation of TCM prescriptions from empirical medicine to evidence-based medicine [16]. Meanwhile, molecular docking, which can be used to identify novel compounds with therapeutic value and predict ligand–target interactions at the molecular level, is a method widely used in drug discovery [17]. These two approaches are highly popular in the current research on the mechanism of TCM.

Considering the theory of 'homotherapy for heteropathy', in this study, we aimed to utilise network pharmacology analysis, molecular docking approach along with some experiments to decipher active compounds of WJD as well as to predict potentially common targets and signaling pathways against pneumonia, COPD, ALI, pulmonary fibrosis, pulmonary tuberculosis and NSCLC.

2. Materials and methods

2.1. Screening the active ingredients in WJD

A website called Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) was used to identify all compounds of four herbs in WJD. The Latin names of herbs (*Rhizoma phragmitis, Coicis semen, Persicae semen* and *Benincasae semen*) were used as key words. Components of WJD were screened based on absorption, distribution, metabolism along with excretion. The mainly active compounds in WJD were selected following the criterion of oral bioavailability (OB) $\geq$20% and drug-likeness (DL) $\geq$0.18.

2.2. Identification of protein targets in WJD

The active compounds of each drug were individually searched in the TCMSP database to obtain the target proteins of the relevant drug compounds. The obtained target protein names were converted to corresponding gene names through the UniProt database. After removing duplicate values, the target genes of WJD were obtained.

2.3. Acquisition of targets of six pulmonary diseases

Pulmonary diseases-associated target genes were retrieved from the GeneCards Database, Therapeutic Target Database (TTD), DisGeNet Database and OMIM Database. The key words 'pneumonia', 'chronic obstructive pulmonary disease', 'acute lung injury', 'pulmonary fibrosis', 'pulmonary tuberculosis' and 'non-small cell lung cancer' were utilized. The target genes obtained in the GeneCards Database were screened by the median of 'Relevance score' to obtain the final target genes. The target genes obtained from each gene database were searched individually through the UniProtKB search function in the UniProt database, and the species was limited to 'human' and 'reviewed' in the search results. At the same time, the names of the corresponding target genes were recorded. After summarizing the target genes obtained from each database, the duplicate values were deleted, and the total number of target genes was recorded.

2.4. Intersection of targets of WJD and six pulmonary diseases

The relevant targets of the active ingredients in WJD and targets of six lung diseases were imported into the VENNY 2.1 online mapping tool to obtain drug-disease intersection targets and corresponding Venn diagrams.

2.5. Establishment of the herb-component-target network

The herbs, active ingredients and intersection targets between WJD and lung diseases were imported into Cytoscape 3.7.2 software to construct six herb-compound-target networks for analysis and visualization.

2.6. Establishment of protein–protein interaction (PPI) network

The targets of WJD in treating six pulmonary diseases were input into STRING 11.5 for PPI analysis. The 'Multiple Proteins' function was used to analyse; '*Homo sapiens*' was set as the species; the confidence level $\geq$0.400 was selected; and the network display mode was shown as 'interactive svg'. The free nodes were hidden, the tsv data file was downloaded and the data were imported to Cytoscape 3.7.2 software for data visualization analysis. Furthermore, the degree value was calculated, and the 'Visualize Parameters' function of Cytoscape was used to visualize the important nodes of the PPI network. The top ten targets of each lung disease treated with WJD were screened out by referring to the degree value.

2.7. GO biological function and KEGG signaling pathway enrichment analysis

The Metascape database was used to conduct GO biological function and KEGG signaling pathway enrichment analyses on the common targets between WJD and pulmonary diseases. The biological species was selected as *Homo sapiens*, and the threshold was set as $P \leq 0.01$. The important enrichment results were visualized and analyzed through Bioinformatics and ImageGP online mapping websites. The output GO enrichment bar charts showed the enrichment results of biological process (BP), cell composition (CC) and molecular function (MF). Cytoscape 3.7.2 was used to calculate the degree value of each enriched pathway, and the pathway-target networks as well as KEGG clustering heat map were obtained after visualization.

2.8. Molecular docking

The 2D molecular structures of the key active components involved in the core therapeutic targets in six lung diseases were obtained through PubChem and then imported into Chem3D to set Minimum RMS Gradient: 0.0100 for molecular energy minimization processing. Small molecule compound files were exported in mol2 format. Then, in the UniProt database, the core target proteins were downloaded, and suitable protein structures were selected. In the PDB database, the secondary structures of the target protein ligands with high structural similarity to the key active components and high resolution were downloaded. Finally, after ligand extraction, hydrogenation, along with water extraction, the docking between small molecules and therapeutic targets was performed by using the Surflex-Dock module of Sybyl-X 2.0.0 and the Surflex-Dock Geom (SFXC) program. The original threshold was set as 0.50, and the original expansion was set as 0. The total scores were calculated by Surflex-Dock to determine the binding activity between small molecule compounds and therapeutic targets. The molecular docking pattern diagrams were also presented.

2.9. Mouse xenograft assay and flow cytometry assay

Freeze-dried powder of WJD was manufactured by ourselves and fingerprint spectrums were finished. The xenograft NSCLC mouse model was established and pharmaceutical effect of WJD was evaluated. These experimental protocols could refer to our previous

article [14]. In brief, C57BL/6J mice were injected with LLC cells subcutaneously (5×10^5 cells/100 μl serum-free cell culture medium). Mice were randomized into two groups including WJD group (i.g. daily, 1071.18 mg/kg) and control group (i.g. daily, normal saline). After the end of the animal experiment (15 days), whole blood was collected from the eyeball of mice and was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was removed and the sediment left was the blood cell. Antibody expressions on the surface of cells were detected, including CD3, CD45, CD4, CD8, CD19, NK1.1, and PD-1 (purchased from Biolegend). Blood cells were dyed for 30 min at 4 °C, out of light. After 30 min, 500 μl of red blood cell lysis buffer (purchased from Sigma Aldrich) was added into each sample, swirling for a few seconds. Keeping stand for 10 min before the solution became clear and then the solution was centrifuged at 3000 rpm for 3 min at 4 °C. The supernatant was removed and the sediment left was taken to be detected by the BD FASC AariaIII flow cytometry.

2.10. Real-time PCR

After the animal experiment, tumors were collected. Tumor's total RNA was extracted by using TRIzol reagent (purchased from Invitrogen). Then, RNA was reversely transcribed to cDNA by using a kit bought from TOYOBO. The mRNA expression levels of critical targets were detected by ViiA™7. Sequences of PCR primers were listed in Table 1.

2.11. Statistical analysis

All results represented data in three independent experiments and were presented as mean ± SE. Results were plotted via GraphPad Prism 7.0 software. The data about immune function were analyzed by using the FlowJo Software Version 10. The data about mRNA expression were calculated in $2^{-\triangle\triangle CT}$ method and analyzed by *t*-test method. *P-value <0.05, **P-value<0.01 and ***P-value <0.001 were considered statistically significant difference.

3. Results

3.1. Active compounds of WJD

According to the criterion, 46 mainly active compounds were selected and shown in Table 2. These components primarily originated from *Rhizoma Phragmitis* (4 compounds), *Coicis Semen* (11 compounds), *Persicae Semen* (28 compounds) and *Benincasae Semen* (3 compounds).

3.2. Construction of the herb-component-target network

After searching the TCMSP database, 81 target genes of WJD were obtained. After summarizing the target genes obtained from the GeneCards Database, Therapeutic Target Database, DisGeNet Database and OMIM Database, a total of 1973 possible targets of pneumonia, 2977 possible targets of COPD, 1729 possible targets of ALI, 2033 possible targets of pulmonary fibrosis, 1944 possible targets of pulmonary tuberculosis and 1184 possible targets of NSCLC were obtained **(Table 3)**. A total of 270 targets were the same among the six lung diseases, indicating the possibility of intervening by treating different diseases with the same treatment. The intersection of the targets in six lung diseases and WJD was determined to obtain the therapeutic targets of WJD for these pulmonary diseases. Venn diagrams exhibited that the overlapping targets of pneumonia, COPD, ALI, pulmonary fibrosis, pulmonary tuberculosis, NSCLC and WJD were 28, 45, 47, 31, 27, and 29, respectively. These values demonstrated that WJD had certain regulatory effects on these lung diseases (Fig. 1A-F). Furthermore, six herb-compound-target networks were built by Cytoscape for data visualization (Fig. 2A-F).

3.3. PPI network construction and top target identification

The magnitude of the degree value represents the high or low position occupied in the interaction of the targets. Table 4 showed the top ten targets amongst six pulmonary diseases treated with WJD, respectively. PPI networks were shown in Fig. 3A-F. The color transitioning from red to yellow represented the degree value from large to small. Among them, the commonly key targets of six

Table 1
Sequences of PCR primers.

Gene Name (mouse)	Primer Name	Sequence (5'—3')
JUN	JUN-Forward	CAGTCCAGCAATGGGCACATCA
	JUN-Reverse	GGAAGCGTGTTCTGGCTATGCA
CASP3	CASP3-Forward	GGAGTCTGACTGGAAAGCCGAA
	CASP3-Reverse	CTTCTGGCAAGCCATCTCCTCA
PTGS2	PTGS2-Forward	GCGACATACTCAAGCAGGAGCA
	PTGS2-Reverse	AGTGGTAACCGCTCAGGTGTTG
ACTIN	ACTIN-Forward	CATTGCTGACAGGATGCAGAAGG
	ACTIN-Reverse	TGCTGGAAGGTGGACAGTGAGG

Table 2
Basic information of mainly active compounds of Weijing decoction.

Mol ID	Chemical component	OB/%	DL	Herb
MOL000359	Sitosterol	36.91	0.75	*Coicis semen*
MOL000449	Stigmasterol	43.83	0.76	*Coicis semen*
MOL000508	Friedelin	29.16	0.76	*Coicis semen*
MOL000953	CLR	37.87	0.68	*Coicis semen*
MOL001323	Sitosterol alpha1	43.28	0.78	*Coicis semen*
MOL001494	Mandenol	42	0.19	*Coicis semen*
MOL001884	Omaine	26.6	0.51	*Coicis semen*
MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	33.55	0.42	*Coicis semen*
MOL002882	[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate	34.13	0.3	*Coicis semen*
MOL008118	Coixenolide	32.4	0.43	*Coicis semen*
MOL008121	2-Monoolein	34.23	0.29	*Coicis semen*
MOL001315	Campesterol-3-O-β-D-glucopyranoside	20.49	0.67	*Persicae semen*
MOL001318	β-sitosterol-3-(6-palmitoyl)glucopyranoside	26.07	0.18	*Persicae semen*
MOL001323	Sitosterol alpha1	43.28	0.78	*Persicae semen*
MOL001325	Campesterol-3-O-β-D-(6-O-palmityl)glucopyranoside	25.65	0.19	*Persicae semen*
MOL001328	2,3-didehydro GA70	63.29	0.5	*Persicae semen*
MOL001329	2,3-didehydro GA77	88.08	0.53	*Persicae semen*
MOL001339	GA119	76.36	0.49	*Persicae semen*
MOL001340	GA120	84.85	0.45	*Persicae semen*
MOL001342	GA121-isolactone	72.7	0.54	*Persicae semen*
MOL001343	GA122	64.79	0.5	*Persicae semen*
MOL001344	GA122-isolactone	88.11	0.54	*Persicae semen*
MOL001348	Gibberellin 17	94.64	0.49	*Persicae semen*
MOL001349	4a-formyl-7alpha-hydroxy-1-methyl-8-methylidene-4aalpha,4bbeta-gibbane-1alpha,10beta-dicarboxylic acid	88.6	0.46	*Persicae semen*
MOL001350	GA30	61.72	0.54	*Persicae semen*
MOL001351	Gibberellin A44	101.61	0.54	*Persicae semen*
MOL001352	GA54	64.21	0.53	*Persicae semen*
MOL001353	GA60	93.17	0.53	*Persicae semen*
MOL001355	GA63	65.54	0.54	*Persicae semen*
MOL001358	Gibberellin 7	73.8	0.5	*Persicae semen*
MOL001360	GA77	87.89	0.53	*Persicae semen*
MOL001361	GA87	68.85	0.57	*Persicae semen*
MOL001362	GA95	20.01	0.49	*Persicae semen*
MOL001368	3-O-p-coumaroylquinic acid	37.63	0.29	*Persicae semen*
MOL001371	Populoside_qt	108.89	0.2	*Persicae semen*
MOL000295	Alexandrin	20.63	0.63	*Persicae semen*
MOL000296	Hederagenin	36.91	0.75	*Persicae semen*
MOL000358	Beta-sitosterol	36.91	0.75	*Persicae semen*
MOL000493	Campesterol	37.58	0.71	*Persicae semen*
MOL000449	Stigmasterol	43.83	0.76	*Benincasae semen*
MOL000359	Sitosterol	36.91	0.75	*Benincasae semen*
MOL007344	Tryptophane	28.55	0.24	*Benincasae semen*
MOL001884	Omaine	26.6	0.51	*Rhizoma phragmitis*
MOL002083	Tricin	27.86	0.34	*Rhizoma phragmitis*
MOL000263	Oleanolic acid	29.02	0.76	*Rhizoma phragmitis*
MOL000449	Stigmasterol	43.83	0.76	*Rhizoma phragmitis*

OB, oral bioavailability; DL, drug-likeness.

Table 3
The number of target genes for six pulmonary diseases in the databases.

Disease name	Genecards	OMIM	DisGeNet	TTD	Total number
Pneumonia	1018	0	953	2	1973
Chronic obstructive pulmonary disease	1521	141	1268	47	2977
Acute lung injury	1518	117	91	3	1729
Pulmonary fibrosis	1156	62	810	5	2033
Pulmonary tuberculosis	1548	66	330	0	1944
Non-small cell lung cancer	944	171	30	39	1184

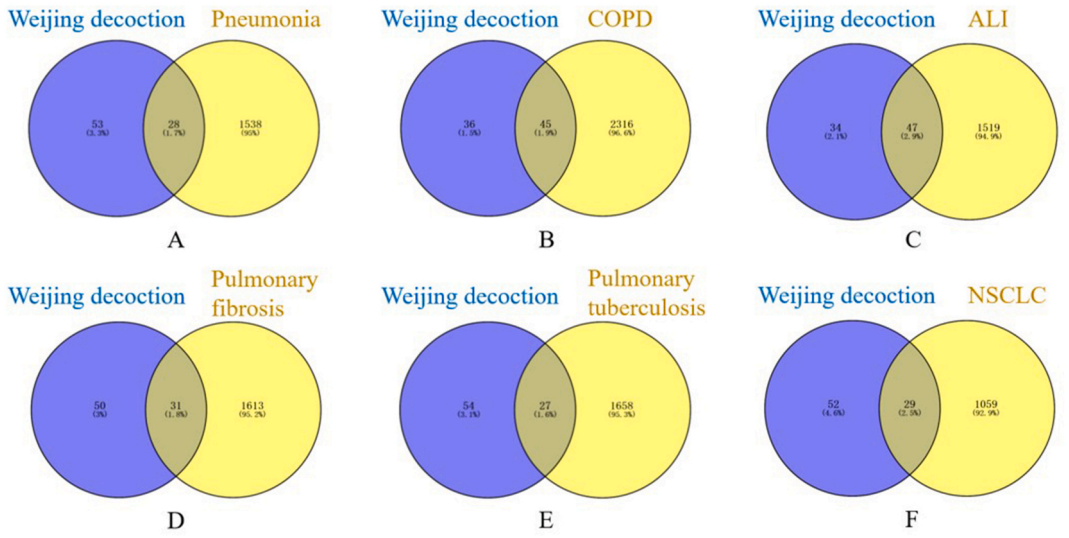

Fig. 1. Venn diagrams about overlapping targets between Weijing decoction-related targets and pulmonary disease-related targets. (A) Weijing decoction and pneumonia (B) Weijing decoction and COPD (C) Weijing decoction and ALI (D) Weijing decoction and pulmonary fibrosis (E) Weijing decoction and pulmonary tuberculosis (F) Weijing decoction and NSCLC.

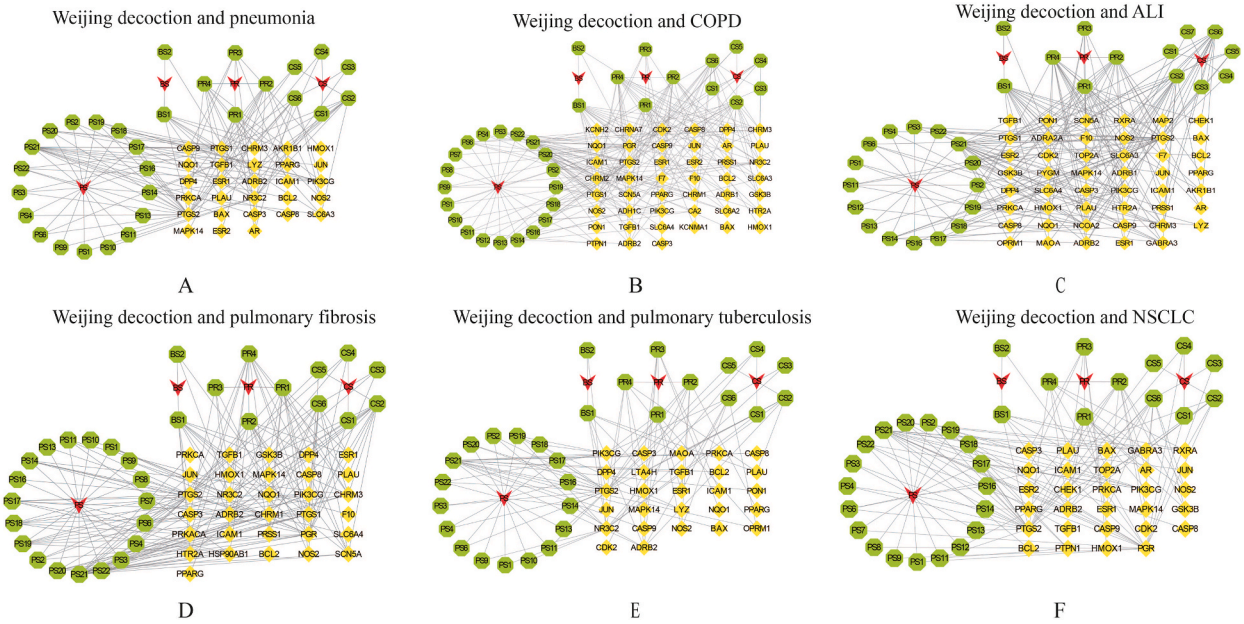

Fig. 2. Herb-compound-target networks. The red arrow shapes represented the herbs in Weijing decoction. The green octagons represented the active compounds in each herb. Meanwhile, the yellow rhombuses represented the overlapping targets between Weijing decoction-related targets and pulmonary disease-related targets. (A) Weijing decoction and pneumonia (B) Weijing decoction and COPD (C) Weijing decoction and ALI (D) Weijing decoction and pulmonary fibrosis (E) Weijing decoction and pulmonary tuberculosis (F) Weijing decoction and NSCLC

diseases included PTGS2, PPARG, MAPK14, CASP3, CASP8 and JUN, indicating that these six targets played key roles in WJD in the treatment of lung diseases. In particular, the top three targets, namely, JUN, CASP3 and PTGS2, were the most critical.

3.4. GO biological function and KEGG pathway enrichment analysis

To further understand the overlapping genes between WJD and pulmonary diseases, Gene Ontology enrichment analysis was conducted. The enrichment results showed that the main biological process, cellular component and molecular function were similar in the regulation of the six pulmonary diseases by WJD. The key biological processes included the cellular response to organic cyclic

Table 4
The core targets of Weijing decoction anti-pulmonary diseases.

Pneumonia		Chronic obstructive pulmonary disease		Acute lung injury	
Targets	Degree	Targets	Degree	Targets	Degree
CASP3	19	JUN	22	JUN	23
PTGS2	19	CASP3	20	CASP3	23
JUN	18	PTGS2	20	PPARG	21
PPARG	16	ESR1	18	PTGS2	21
ESR1	14	PPARG	18	ESR1	20
MAPK14	13	MAPK14	15	CASP8	16
CASP8	13	CASP8	15	MAPK14	13
HMOX1	13	HMOX1	14	GSK3B	13
ICAM1	12	GSK3B	12	PRKCA	11
CASP9	10	CDK2	11	NOS2	11
Pulmonary fibrosis		Pulmonary tuberculosis		Non-small cell lung cancer	
Targets	Degree	Targets	Degree	Targets	Degree
JUN	18	JUN	17	JUN	23
CASP3	17	CASP3	17	CASP3	21
PPARG	16	PPARG	16	PTGS2	20
PTGS2	16	PTGS2	16	ESR1	19
ESR1	15	HMOX1	12	PPARG	17
MAPK14	12	CASP8	12	CASP8	16
GSK3B	11	MAPK14	11	MAPK14	15
CASP8	10	ICAM1	11	CDK2	13
HSP90AB1	9	NOS2	10	CASP9	13
PGR	9	TGFB1	10	GSK3B	13

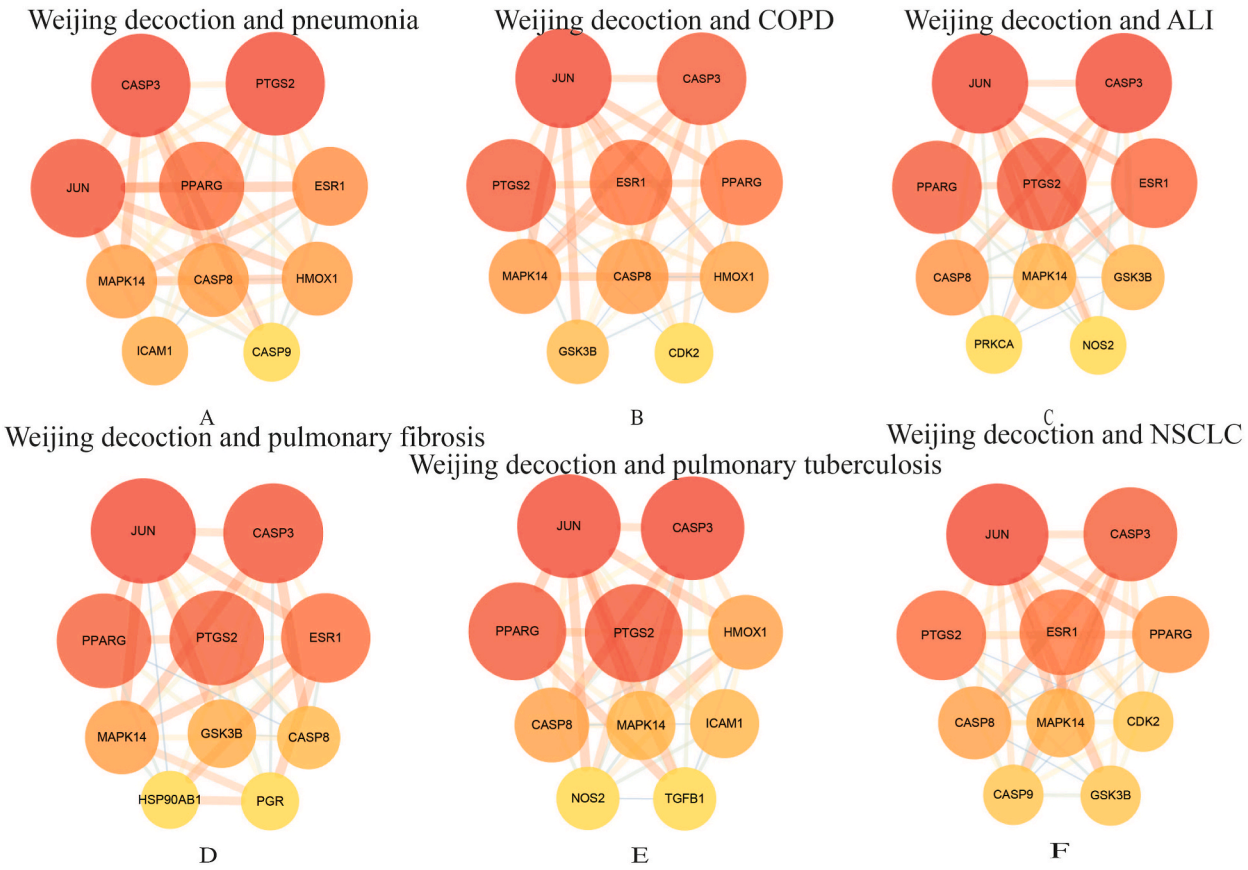

Fig. 3. PPI networks about the core targets of Weijing decoction anti-pulmonary diseases. The color transitioning from red to yellow represented the degree value from large to small. (A) Weijing decoction treated for pneumonia (B) Weijing decoction treated for COPD (C) Weijing decoction treated for ALI (D) Weijing decoction treated for pulmonary fibrosis (E) Weijing decoction treated for pulmonary tuberculosis (F) Weijing decoction treated for NSCLC

compound and blood circulation. The key cellular components included the membrane raft and organelle outer membrane. Meanwhile, the key molecular functions included the protein homodimerization activity and protein domain specific binding (Fig. 4A-F).

In order to cluster the major effects related to WJD anti-lung diseases, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted. The top ten pathways of each disease enrichment results were visualized to obtain pathway-target networks (Fig. 5A-F). The 21 top-ranked and shared pathways in six lung diseases were then selected to draw a KEGG clustering heat map (Fig. 6). The results exhibited that WJD had extensive pharmacological regulation, involving pathways related to cancer, inflammation, infection, hypoxia, immunity and so on. Hence, WJD could play a vital role in anti-pulmonary diseases via coordinating different pathways.

3.5. Molecular docking of active components and key targets

Molecular docking enables the prediction of ligand conformation within appropriate target binding sites in a considerable range of accuracy. The quantitative prediction of binding energy is carried out by the molecular docking algorithm, and the total scores are ranked based on the binding force of ligand-compound. According to the PPI network, the three most critical therapeutic targets for six lung diseases were JUN, CASP3 and PTGS2. The secondary structures of the therapeutic targets were selected through the UniProt database and PDB database. The secondary structure number selected by JUN was 5FV8; selected by CASP3 was 3KJF; and selected by PTGS2 was 5F1A. The key ingredients in WJD to treat lung diseases were chosen for molecular docking with these three key targets. The key active ingredients in WJD with better binding effects were beta-sitosterol, tricin and stigmasterol. The affinity of the small molecules to the targets was assessed by the total scores calculated by the Surflex-Dock module in Sybyl-X 2.0.0. It is generally thought that the score larger than 4.0 shows that the molecule has a certain binding effect with the target. The score larger than 5.0 demonstrates a good binding effect. At the same time, the score larger than 7.0 manifests a strong binding effect [18]. Table 5 exhibited total docking scores of the binding activity between three key compounds and three core targets. Beta-sitosterol, tricin, and stigmasterol mostly had certain binding effects with JUN and CASP3. Both beta-sitosterol and stigmasterol had scores higher than 5.0 with PTGS2, showing good binding activity. More importantly, tricin had a score higher than 7.0 with PTGS2, suggesting strong binding activity. Then, the molecular docking pattern diagram (Fig. 7 A1-7C3) indicated that beta-sitosterol, tricin, and stigmasterol stably bound to a variety of active sites on the JUN, CASP3 and PTGS2 target proteins.

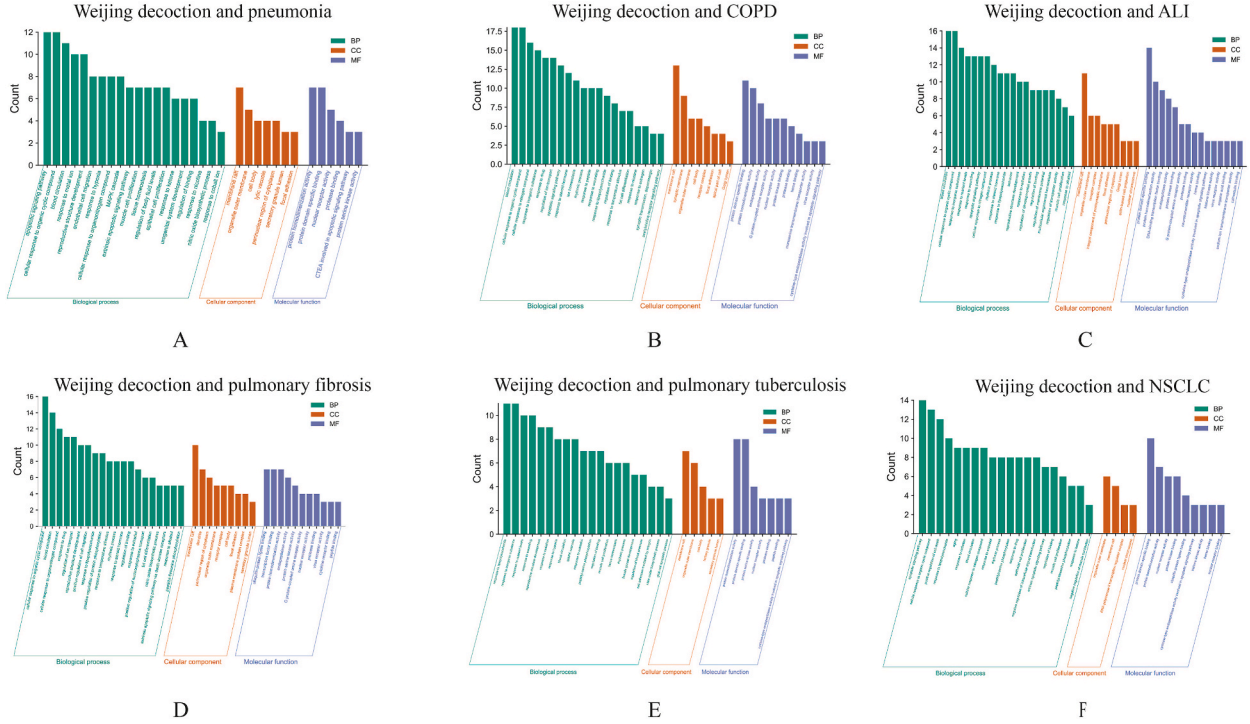

Fig. 4. The GO enrichment analysis of the overlapping genes between Weijing decoction and pulmonary diseases. The green histogram represented the biological process. The red histogram represented the cellular component. The blue histogram represented the molecular function. (A) Weijing decoction treated for pneumonia (B) Weijing decoction treated for COPD (C) Weijing decoction treated for ALI (D) Weijing decoction treated for pulmonary fibrosis (E) Weijing decoction treated for pulmonary tuberculosis (F) Weijing decoction treated for NSCLC

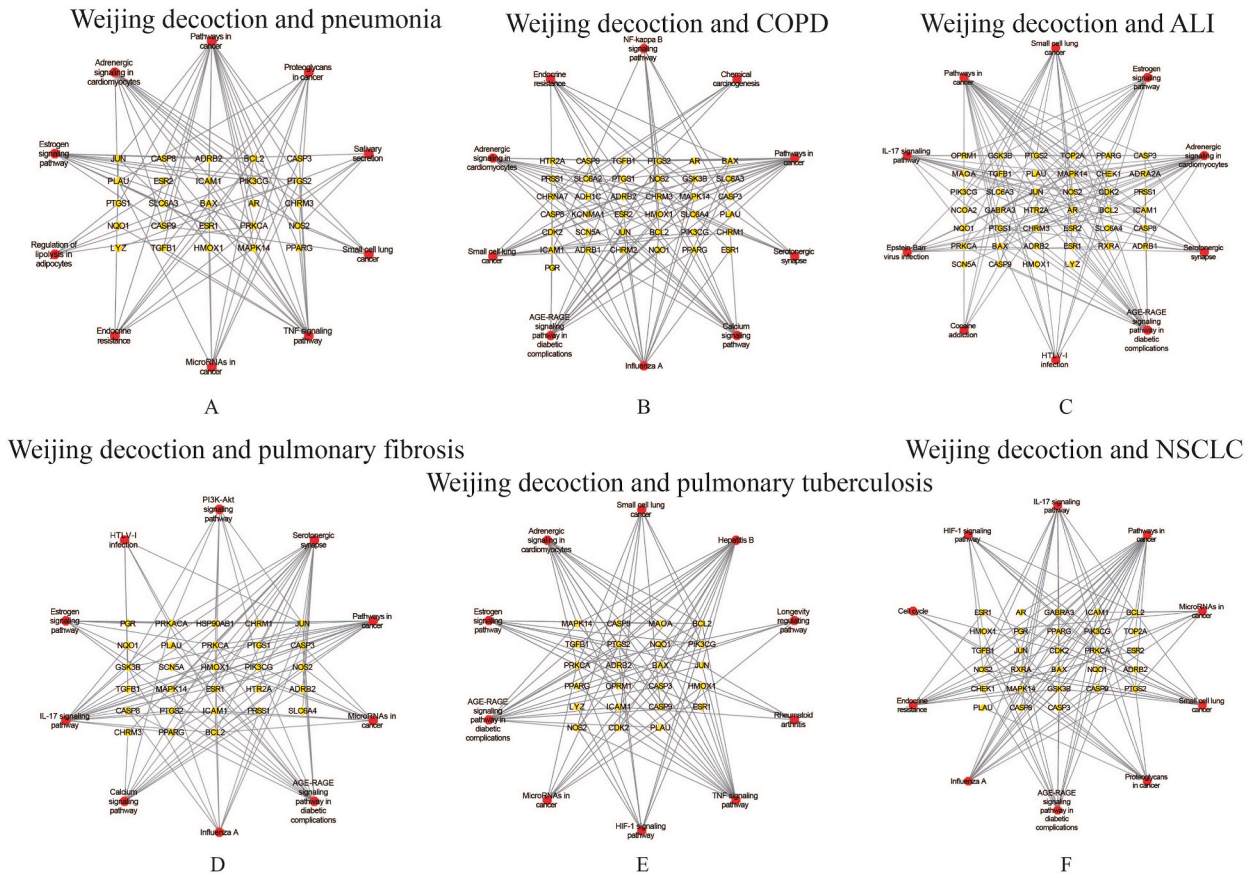

Fig. 5. Target–pathway network of KEGG enrichment analysis of the overlapping genes between Weijing decoction and pulmonary diseases. The yellow rhombuses represented the hub overlapping targets while the red octagons represented the related pathways. (A) Weijing decoction treated for pneumonia (B) Weijing decoction treated for COPD (C) Weijing decoction treated for ALI (D) Weijing decoction treated for pulmonary fibrosis (E) Weijing decoction treated for pulmonary tuberculosis (F) Weijing decoction treated for NSCLC

3.6. WJD could regulate the immune function

In our previous research, we have found that WJD was able to inhibit NSCLC growth obviously without serious toxicity and to prolong the survival time [14]. Thus, the xenograft NSCLC mouse model was chosen for this exploration again. In order to understand whether the WJD could regulate the immune function or not, the flow cytometry was used to verify this. Fig. 8A and B were representative flow cytometry analysis pictures between the control group and the WJD group. As were shown in Fig. 9A-C, the percentages of $CD4^+/CD8^+$, B lymphocytes as well as NK cells (natural killer cells) were significantly higher in the WJD group in comparison with the control group. These data indicated that WJD could enhance the immunity. At the same time, the expressions of PD-1 on $CD4^+$ and $CD8^+$ T cells, B lymphocytes as well as NK cells were significantly higher in WJD group in comparison with the control group, which were exhibited in Fig. 9D-G, indicating that WJD might be involved in regulating the PD-1/PD-L1 pathway along with the re-activation of T cells, B cells and NK cells.

3.7. WJD could decrease the mRNA expression level of critical genes in tumors

Real-time PCR assay was applied to verify WJD's effect on the mRNA expression levels of three core targets which were mentioned in above network pharmacology results. In comparison with the control group, the mRNA expressions of JUN and CASP3 in WJD group were down-regulated by 2.04-fold and 2.78-fold, respectively ($P < 0.01$). Especially, in comparison with the control group, the mRNA expression of PTGS2 in WJD group was down-regulated by about 20.0-fold ($P < 0.001$). These results demonstrated that WJD played important roles in regulating gene JUN, CASP3 and PTGS2 (Fig. 10A-C).

4. Discussion

As widespread diseases, pulmonary diseases have increasingly serious effects on physical health, and they may even lead to death. Although some Western medicines are effective, they often induce side effects and may not be universally available for various types of

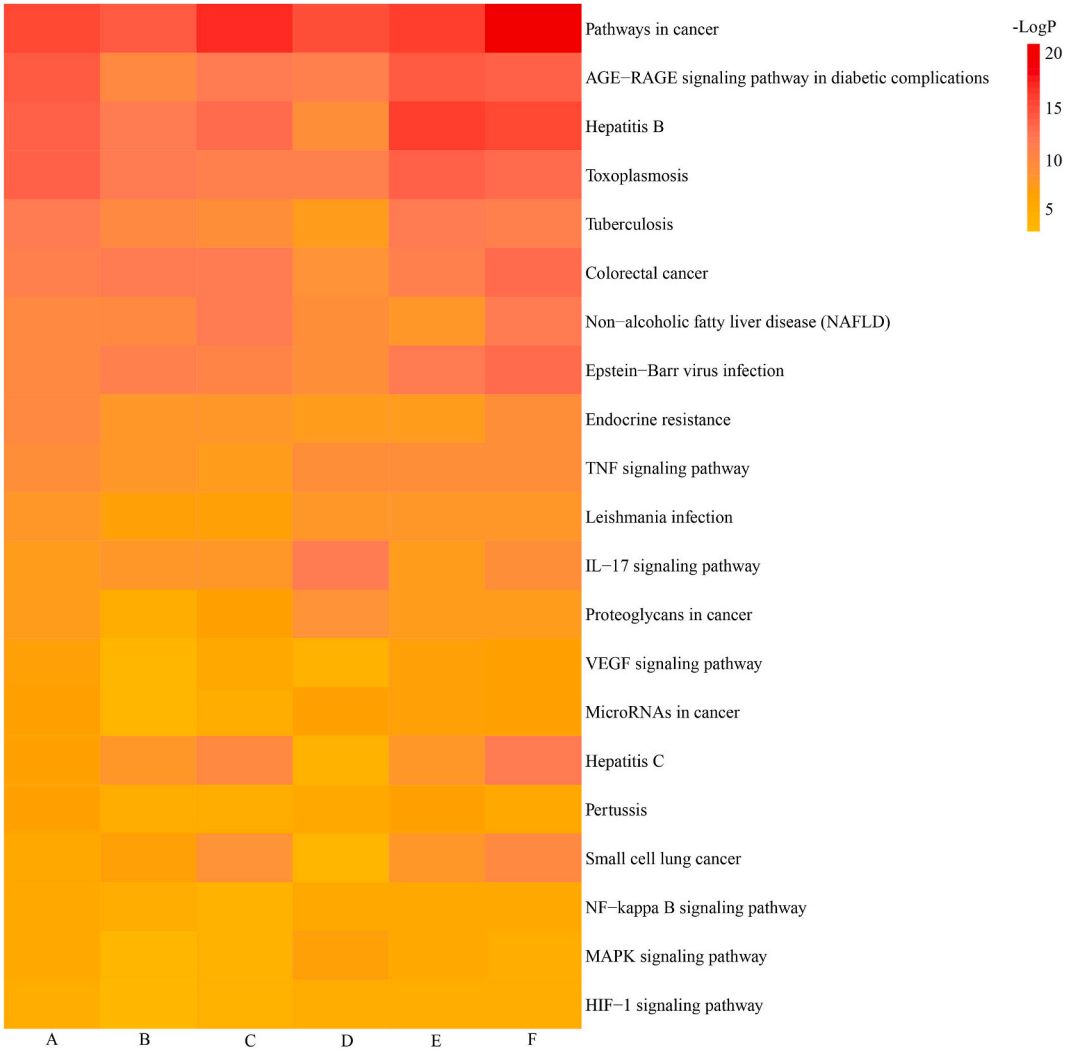

Fig. 6. KEGG clustering heat map of Weijing decoction for six lung diseases. As the P value changed from large to small, the corresponding color altered from yellow to red. (A) Weijing decoction treated for pneumonia (B) Weijing decoction treated for COPD (C) Weijing decoction treated for ALI (D) Weijing decoction treated for pulmonary fibrosis (E) Weijing decoction treated for pulmonary tuberculosis (F) Weijing decoction treated for NSCLC

Table 5
The docking scores of key compounds and core targets.

Mol ID	Compound	Herb	Target	Total Score
MOL000358	Beta-sitosterol	*Persicae semen*	JUN	4.85
MOL002083	Tricin	*Rhizoma phragmitis*	JUN	3.45
MOL000449	Stigmasterol	*Coicis semen; Benincasae semen; Rhizoma phragmitis*	JUN	4.08
MOL000358	Beta-sitosterol	*Persicae semen*	CASP3	4.36
MOL002083	Tricin	*Rhizoma phragmitis*	CASP3	4.23
MOL000449	Stigmasterol	*Coicis semen; Benincasae semen; Rhizoma phragmitis*	CASP3	4.79
MOL000358	Beta-sitosterol	*Persicae semen*	PTGS2	6.73
MOL002083	Tricin	*Rhizoma phragmitis*	PTGS2	7.29
MOL000449	Stigmasterol	*Coicis semen; Benincasae semen; Rhizoma phragmitis*	PTGS2	6.75

lung diseases. In the field of TCM, the theory of 'homotherapy for heteropathy' is well known. It is a diagnosis and treatment concept with Chinese characteristics and is expected to be verified by modern medicine under the guidance of the principle of syndrome differentiation and treatment of TCM. The human body is a whole. Starting from the overall syndrome differentiation, different diseases can be identified as the same syndrome if the pathogenesis is the same. The same method can also be used for treatment. For

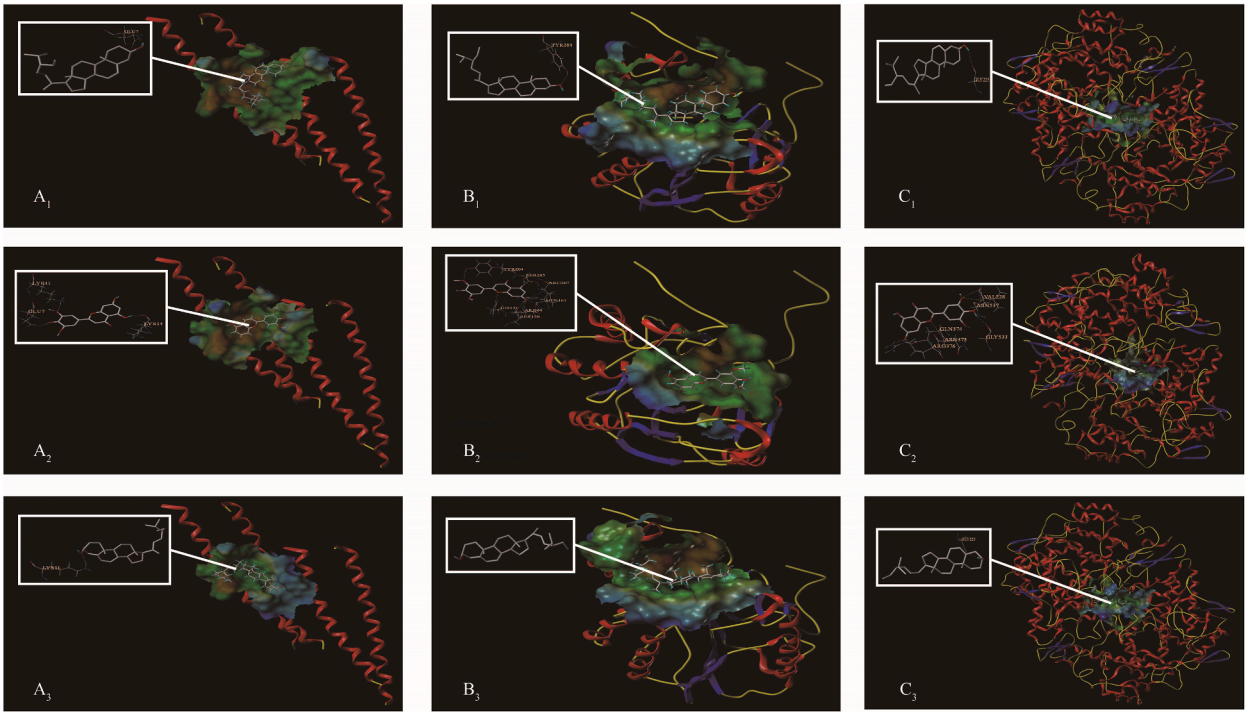

Fig. 7. Molecular docking pattern diagrams about key compounds and core targets. (A1) JUN and beta-sitosterol (A2) JUN and tricin (A3) JUN and stigmasterol (B1) CASP3 and beta-sitosterol (B2) CASP3 and tricin (B3) CASP3 and stigmasterol (C1) PTGS2 and beta-sitosterol (C2) PTGS2 and tricin (C3) PTGS2 and stigmasterol.

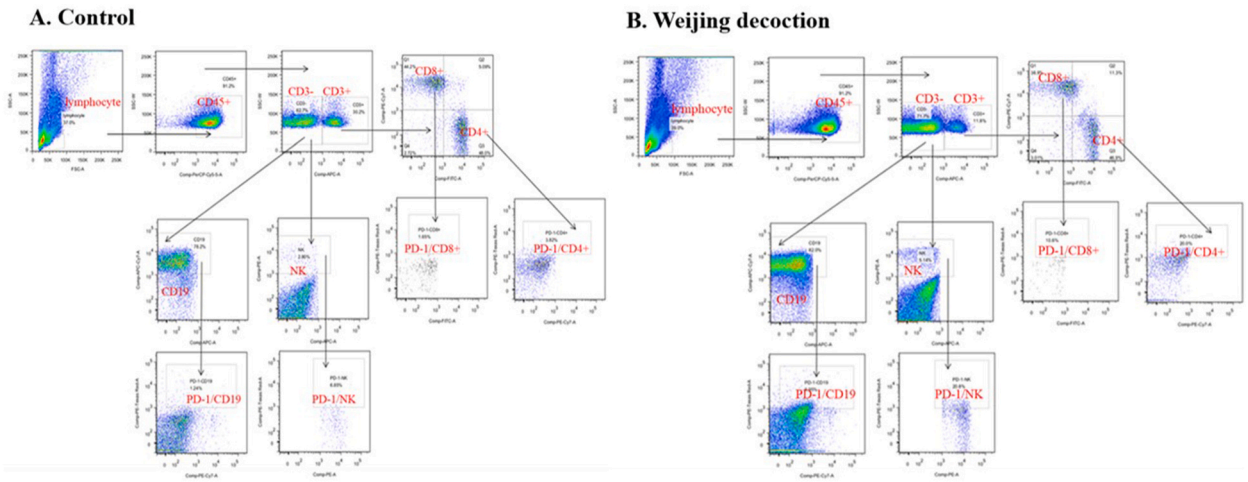

Fig. 8. Representative flow cytometry analysis between the control group and the Weijing decoction group.

example, diabetes retinopathy, ischemic coronary heart disease and acute altitude hypoxia disease are all progressive diseases involving multiple systems caused by Qi stagnation and blood stasis. At the same time, modern medical research believes that these three diseases have common pathological characteristics, that is, they are all complex molecular networks of disease, which are composed of oxidative stress and inflammatory reaction caused by tissue ischemia and hypoxia, mitochondrial energy supply disorder of local tissue cells, and blood circulation disorder. Therefore, the three diseases can be treated with Compound Danshen Dropping Pills according to the macroscopic syndrome differentiation of TCM and the microscopic study of modern medicine. By analogy, pulmonary diseases such as pneumonia, COPD, ALI, pulmonary fibrosis, pulmonary tuberculosis and NSCLC all have similar pathogenesis, which may be treated with one Chinese formula. Currently, WJD can be used to treat COPD, NSCLC and even COVID-19 in clinical settings. There are few basic studies on the treatment of diseases with WJD. In the previous research, Dr. Liu has conducted the

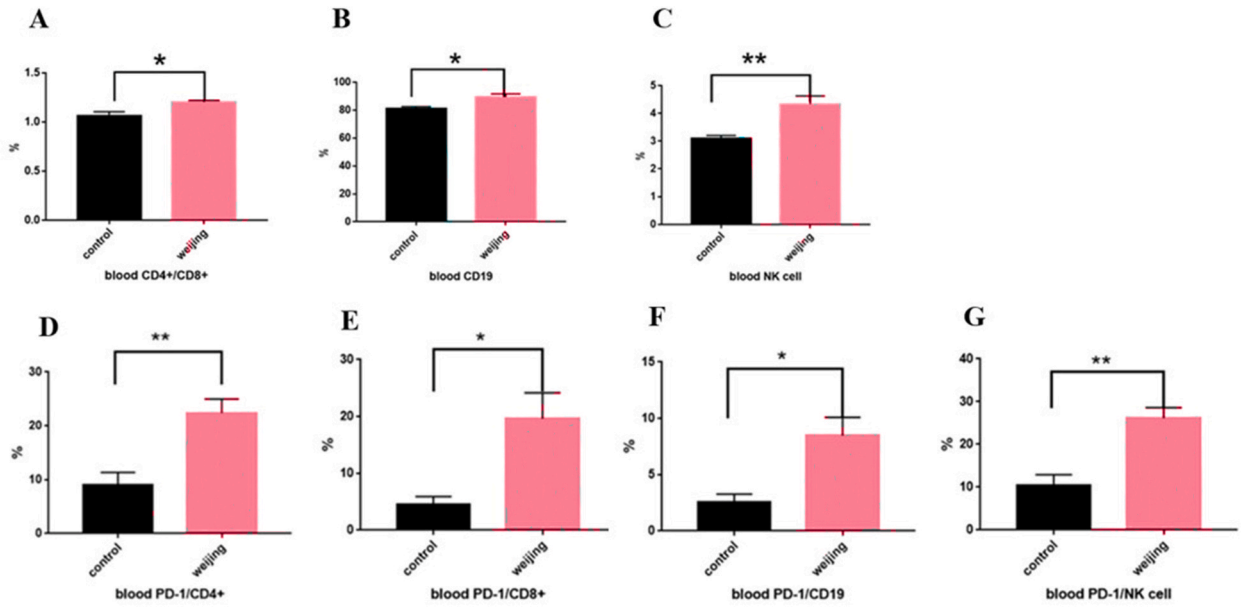

Fig. 9. WJD regulated the immune system and increased the expression of PD-1 on $CD4^+$ and $CD8^+$T cells, B lymphocytes as well as NK cells. (A–C) Bar graph showed the percentages of $CD4^+/CD8^+$, B lymphocytes as well as NK cells between the control group and WJD group. (D–G) Bar graph showed the expressions of PD-1 on $CD4^+$ and $CD8^+$ T cells, B lymphocytes as well as NK cells between the control group and WJD group. (*P < 0.05, **P < 0.01).

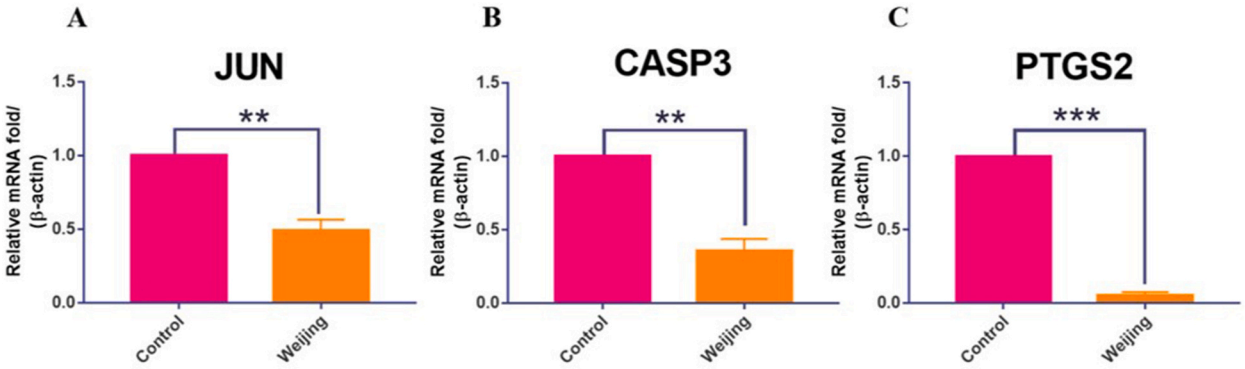

Fig. 10. WJD downregulated mRNA expression levels of JUN, CASP3 and PTGS2 gene in tumors. (**P < 0.01, ***P < 0.001).

meta-analysis to evaluate the effectiveness of the combination of pharmacotherapy and WJD in treating acute exacerbation of chronic obstructive pulmonary disease [13]. What' more, Dr. Xiong found that through inhibiting the PI3K/AKT signaling pathway, WJD combined with MaiMenDong decoction and cisplatin could suppress the growth of A549 human lung cancer cells [19]. Besides, our group demonstrated that WJD inhibited the growth of LLC lung cancer cells mainly via PRKCA/SPHK/S1P signaling pathway in previous study [14].

TCM includes many theories, such as essential qi theory, yin-yang theory, five phase theory, theory of visceral manifestations, meridian and collateral theory and so on. Pulmonary diseases are mainly located in the lung system, which is not completely the same as the lung in modern medicine. The principle of treating lung diseases with WJD can be explained by the theory of visceral manifestations and viscera syndrome differentiation. The main physiological function of lung system is to regulate water passage as well as govern Qi and respiration. The lung regulates the whole body's Qi through respiratory movement, thus promoting blood circulation. If there is abnormality, it will lead to metabolic disorders. Pathological products such as phlegm and blood stasis will appear. In the concept of TCM, most lung diseases are caused by phlegm and blood stasis. The function of WJD is to remove heat from the lung, dissolve phlegm, remove blood stasis and expel pus. WJD is used to treat the symptoms of cough, much phlegm, chest pain, vomiting pus and fever, which are consistent with the symptoms of pneumonia, COPD, ALI, pulmonary fibrosis, tuberculosis, and lung cancer in modern medicine. Therefore, WJD can be used to treat a series of pulmonary diseases under the support of TCM theory.

In this work, we first explored common key compounds, core targets and main pathways of WJD in the treatment of six different

lung diseases by combining network pharmacology, molecular docking and some preliminary experiments. A schematic diagram to show the function of predicted major targets and active components in the six pulmonary diseases has been added for illustrating how 'homotherapy for heteropathy' works in this case (Fig. 11).

In this study, KEGG pathway enrichment analysis demonstrated that the core targets were mainly enriched in the cancer pathway, AGE−RAGE signaling pathway, virus infection pathway, TNF signaling pathway, IL−17 signaling pathway, VEGF signaling pathway, NF−kappa B signaling pathway, MAPK signaling pathway and HIF−1 signaling pathway, which were associated with immune dysfunction, inflammatory reaction, angiogenesis, infection, hypoxia induction and oxidative stress in some pulmonary diseases, such as pneumonia, COPD, ALI, pulmonary fibrosis, pulmonary tuberculosis and NSCLC. Results of the PPI network indicated that PTGS2, PPARG, MAPK14, CASP3, CASP8 and JUN were considered common hub targets of the above six lung diseases. In particular, JUN, CASP3 and PTGS2 were the most critical. These hub genes were tightened with the above corresponding pathways. First and foremost, JUN is a protein coding gene called Jun Proto-Oncogene. It is encoded by the related gene located on chromosome 1, and the locus is 1p32-p31 [20]. JUN is associated with cell proliferation, cell apoptosis as well as tissue morphogenesis [21]. At the same time, it also interacts with some pathways including TNF signaling pathway, IL−17 signaling pathway, VEGF signaling pathway, MAPK signaling pathway, HIF-1 signaling pathway, cancer pathway, AGE−RAGE signaling pathway and virus infection pathway **(GeneCards database)**. Besides, tumors which are in connection with JUN cover sarcoma, primary cutaneous T-cell lymphoma and NSCLC **(GeneCards database)** [22]. Secondly, the caspase is mainly involved in cell apoptosis, necroptosis, and pyroptosis and inflammation, making it an important target for the therapeutic intervention [23]. CASP3 (caspase-3) is the most widely studied apoptotic protein in the caspase family, and it plays an important role in programmed cell death. It is located on the long arm of chromosome 4 (Q35.1) and plays a key role as an executive-stage caspase during apoptosis. Many genetic alterations of CASP3 are related to various tumors including NSCLC, therefore regulating apoptotic activity in patients with cancer [24]. In addition, in the pathogenesis of COPD, CASP3 also plays a crucial role in cell apoptosis [25]. Moreover, activation of CASP3 triggers the transcription of inflammatory genes [26]. CASP3 is linked with TNF pathway, IL-17 pathway, AGE/RAGE pathway, VEGF pathway, cancer pathway, virus infection pathway and NF−kappa B pathway, which referred in KEGG pathway enrichment analysis **(GeneCards database)**. Thirdly, PTGS2 (Prostaglandin-Endoperoxide Synthase 2) is also known as cyclooxygenase-2 (COX-2), which is in charge of prostaglandin biosynthesis in inflammation **(GeneCards database)**. The PTGS2 gene is particularly associated with promoting inflammation, increasing oxidative stress and developing COPD [27]. Furthermore, overproduction of PTGS2 is observed in NSCLC and is involved in tumor progression by accelerating angiogenesis, metastasis, along with immunosuppression [28,29]. In terms of related KEGG enrichment pathways, PTGS2 is involved in the cancer pathway, VEGF signaling pathway, NF-kappaB signaling pathway, AGE−RAGE signaling pathway, virus infection pathway, TNF signaling pathway, IL−17 signaling pathway and HIF−1 signaling pathway **(GeneCards database)**.

Results of molecular docking demonstrated that the key active ingredients in WJD with better binding effects on core targets were beta-sitosterol, tricin and stigmasterol. Beta-sitosterol ($C_{29}H_{50}O$) is a famous plant sterol existed in many plants including seabuckthorn and wolfberries, playing important roles in multiple signaling pathways such as cell apoptosis, proliferation, angiogenesis and inflammation. Some reports showed that beta-sitosterol has the antioxidant, anti-inflammatory as well as anti-tumor activities [30–32]. Dr. Ramalingam et al. found that oral β-sitosterol reduced renal carcinoma-induced elevations in c-FOS and c-JUN, exhibiting an antiproliferative effect [33]. Dr. Lin et al. demonstrated that β-sitosterol downregulated caspase-3 and caspase-9 protein levels in the hypoxia/reoxygenation-stimulated rat cardiomyocyte cell line (H9c2), exerting a protective effect by suppressing cell apoptosis [34]. Moreover, β-sitosterol attenuates the pro-inflammatory enzyme COX-2 (PTGS2), which can be regarded as a prospective compound to treat inflammatory disorders [35]. Tricin ($C_{17}H_{14}O_7$) is a natural flavonoid compound mainly isolated from *Rhizoma Phragmitis*, wheat and rice, which has antiviral, anti-tuberculous, anti-inflammatory, anti-angiogenic, immunomodulatory, antiulcer, antioxidant along with anti-tumor effects [14,36–41]. Dr. Yan et al. found that Tricin could be used as a molecule for designing the novel c-Jun N-Terminal kinase 1 (JNK1) inhibitor [42]. Tricin is also a component extracted from alfalfa (*Medicago Sativa*). Dr. Grégory

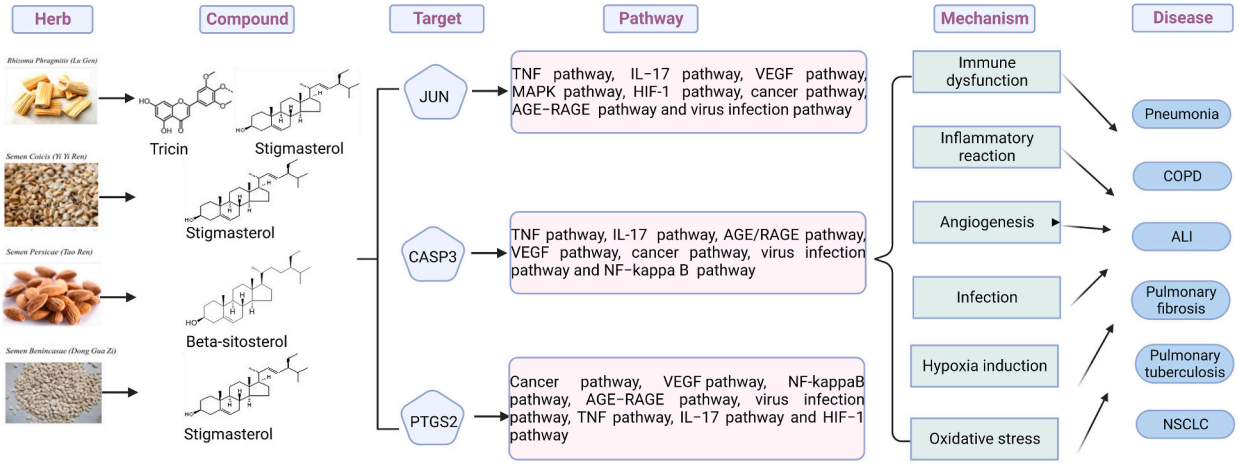

Fig. 11. A schematic diagram to show the function of predicted major targets and active components in the six pulmonary diseases.

et al. found that alfalfa-mediated cancer cell apoptosis was triggered via the mitochondrial pathway, which led to CASP3 activation [43]. On the other hand, treatment with tricin isolated from *Alopecurus Aequalis* obviously downregulates COX-2 (PTGS2) expression, thereby inhibiting the inflammatory response [44]. Stigmasterol ($C_{29}H_{48}O$) is the main phytosterol or plant lipid in various herbs, exerting various roles as an anti-pyretic, anti-tumor, anti-inflammatory and immune-modulating agent [45–48]. Dr. Nazmul et al. demonstrated that stigmasterol enhanced neuronal migration by activating c-JUN N-terminal kinase, then protecting brain development [49]. Stigmasterol can induce the apoptosis of gall bladder cancer cells through CASP3 activation [50]. What's more, stigmasterol is able to relieve inflammation via decreasing the expression of COX-2 (PTGS2), then attenuating cerebral ischemia/reperfusion injury [51].

Traditional Chinese decoction has been widely used in clinical practice for thousands of years and has its unique advantages as well as clinical efficacy. However, with the progress of society and the change of contemporary medication use habits, the use of traditional Chinese decoction shows increasing weakness. For example, taking long time to decoct herbal medicine, inconvenient to carry, short storage time and the poor quality control of TCM makes it difficult to be pushed to the international market. Therefore, exploring the composition and target of TCM formula by modern research methods such as network pharmacology and metabolomics is the first step to transform and develop new drugs. In the future, we will conduct a systematic study on the quality control, component analysis and identification, toxicological mechanism exploration, clinical safety and efficacy evaluation, as well as reform the dosage form, so as to expand the clinical use of TCM formula and open the international market.

5. Limitations

Although we have some understanding of the mechanism of WJD in the treatment of lung diseases, our study still has several limitations. First of all, our results require to be better validated after we get familiar with more animal models of lung diseases, not just the NSCLC model. Next, additional comprehensive databases are needed so that the results of network pharmacology are complete and reliable. Furthermore, a deeper understanding of the mechanism requires the support of multi-omics technology. The characteristics of multi-component and multi-target of TCM compounds provide an effective solution for alleviating the drug resistance and side effects caused by a single target of Western medicine. In the future, we need to conduct research on more traditional Chinese formulas, so that the concept of 'homotherapy for heteropathy' will be widely used.

6. Conclusion

In summary, we predicted and preliminarily verified that the key compounds such as beta-sitosterol, tricin and stigmasterol in WJD acted on core targets including JUN, CASP3 and PTGS2, along with some signaling pathways associated with cancer, immunity, inflammation, infection and hypoxia, in order to treat pulmonary diseases consisting of pneumonia, COPD, ALI, pulmonary fibrosis, pulmonary tuberculosis and NSCLC.

Author contribution statement

Jia-Xin Li; Zhong-Xiao Han: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xin Cheng; Feng-Lin Zhang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jing-Yi Zhang; Zi-Jie Su; Biao-Ping Li; Zhi-Rui Jiang; Run-Ze Li; Ying Xie: Analyzed and interpreted the data; Wrote the paper.

Pei-Yu Yan; Ling Tang; Jia-Shun Yang: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Disclosure statement

All authors have no conflicts of interest to declare.

Acknowledgments

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