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Mengmei Zhang
zunyi yiyao gaodeng zhuanke xuexiao: Zunyi Medical and Pharmaceutical College
Guangcai Li
zunyi yiyao gaodeng zhuanke xuexiao: Zunyi Medical and Pharmaceutical College
Huaqing Hu
Gushi County people's Hospital
Yi Mu
Zunyi Medical University
Yang Li
zunyi yixueyuan dier fushu yiyuan: The Second Affiliated Hospital of Zunyi Medical University
Jihang Luo
Affiliated Hospital of Zunyi Medical University
Yan Tang
Affiliated Hospital of Zunyi Medical University
Guangmin Xu
zunyi yiyao gaodeng zhuanke xuexiao: Zunyi Medical and Pharmaceutical College
Ze Yang (918471604@qq.com)
Zhuhai City People's Hospital https://orcid.org/0000-0003-1898-504X
Xiao Liu
Affiliated Hospital of Zunyi Medical University https://orcid.org/0000-0003-1247-8469

Research Article

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Study on the molecular mechanism of aster tataricus L. f. in the treatment of radiation pneumonitis based on Systems Pharmacology

Mengmei Zhang[1*], Guangcai Li[1*], Huaqing Hu[2], Mu Yi[3], Yang Li[4], Jihang Luo[5], Yan Tang[5], Guangmin Xu[1], Ze Yang[6#], Xiao Liu[4#]

1. Zunyi Medical and Pharmaceutical College, Gui Zhou, Zun Yi, China
2. Gushi County People's Hospital, He Nan, Gu Shi, China
3. Zunyi Medical University, Gui Zhou, Zun Yi, China
4. The Second Affiliated Hospital of Zunyi Medical University, Gui Zhou, Zun Yi, China
5. Affiliated Hospital of Zunyi Medical University, Gui Zhou, Zun Yi, China
6. Zhuhai Hospital Affiliated with Jinan University, Zhu Hai, Guangdong, China

Mail:
Mengmei Zhang
zmmpla@hotmail.com
Guangcai Li
liguangcaizunyi912@126.com
Huaqing Hu
54262178@qq.com
Mu Yi
459210965@qq.com
Yang Li
526193792@qq.com
Jihang Luo
772097377@qq.com
Yan Tang
731788096@qq.com
Guangmin Xu
524396160@qq.com

✉ Ze Yang

918471604@qq.com

✉ Xiao Liu

lx2010zlx@163.com
* All authors are first authors.
# All authors are corresponding authors.

Abstract

Background: The occurrence of radiation pneumonia not only affects the efficacy of radiotherapy, but also seriously threatens the health of patients undergoing radiotherapy for lung cancer. Studies have suggested that feining granule is a potentially effective drug for the treatment of radiation pneumonitis, but its mechanism and the main components of its effect are still unclear. Our study used bioinformatics methods to analyze the main the main drug- aster tataricus L. f. in Feining granules, and study its main targets and pathways in the treatment of radiation

pneumonia.

Methods: Analyzed the effective drug components and targets of aster through the Traditional Chinese Medicine Systems Pharmacology (TCMSP) website. And obtained gene targets related to radiation pneumonia through the website of Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), Genecard (https://www.genecards.org/) and Disgenet (https://www.disgenet.org/home/). Protein–protein interaction (PPI), Go and Kegg analysis of the obtained drugs and gene-related targets were conducted. Verify the effects of small molecule drugs on corresponding targets by conducting molecular docking experiments

Results: A total of 193 targets were identified for 13 molecules of aster tataricus L. f., and 897 genes were identified to be related to radiation pneumonia. Finally, we obtained 111 genes by crossing drug and disease related target genes. After PPI, Go and Kegg analysis, we found TP53, HSP90AA1, RELA, JUN, AKT1, MAPK1, TNF and IL-6 are the most critical genes, and these key genes were most focused on the gene ontologies of DNA-binding transcription factor, RNA polymerase II-specific DNA-binding transcription factor and protein serine/threonine kinase activity, and the pathways of lipids and atherosclerosis, advanced glycation end products and their receptors, and interleukin 17. Through molecular docking experiments, it was found that the small molecules of quercetin and luteolin bind tightly to Rela and jun proteins.

Conclusion: Through this study, we reveal the mechanism of action of aster in the treatment of radiation pneumonia, and provides a basis for the research on the treatment of radiation pneumonia and the development of new pharmaceutical ingredients in aster tataricus L. f. Quercetin and luteolin may be effective small molecules for radiation pneumonitis

Keywords

Lung cancer, radiation pneumonitis, aster tataricus L. f., network pharmacology, traditional Chinese medicine

Introduction

Worldwide, lung cancer is one of the leading causes of death in malignant tumors, and its incidence remains high[1]. Radiotherapy, as one of the main treatment of lung cancer, is a very important method for inoperable patients, especially for locally advanced and partially advanced lung cancer patients[2-4]. However, during the course of radiotherapy for primary lung lesions, the occurrence of radiation pneumonitis often adds a lot of trouble to patients. The incidence of radiation pneumonitis is as high as 30%, and nearly 20% of these patients require oxygen and other ventilation support. To make matters worse, about 2% of these patients will die[5]. This data would be even higher in combination therapies, such as, radiotherapy combined with chemotherapy, immunotherapy and targeted therapy and so on[5, 6]. Combination regimens are confirmed to be effective treatments for improving the prognosis of lung cancer[7]. Therefore, how to reduce the incidence of radiation pneumonitis, and effectively treat it, plays an important role in the treatment of lung cancer.

Chinese traditional medicine have achieved many good outcomes in the treatment of many diseases, such as Artemisinin for malaria, Tripterygium Wilfordii for osteoarthritis etc. Feining granule is a traditional Chinese medicine patent prescription. It is used to treat lung infection, bronchitis, asthmatic bronchitis, acute respiratory tract infection and so on. Our previous study and another study suggest that feining granule is a potentially effective drug for the treatment of radiation pneumonitis[8, 9]. However, it is unknown which small molecule in Feining Granules plays a key role in the treatment of radiation pneumonitis. The main ingredient of feining granule is aster tataricus L. f. (Chinese name as 紫菀, pinyin as ziwan), which has the effect of clearing heat and removing phlegm, relieving cough and asthma. So it is very important to study the mechanism of aster tataricus L. f. in the treatment of radiation pneumonitis.

Network pharmacology is a new subject based on the theory of system biology, which analyses the network of biological system and selects specific signal Nodes (Nodes) to design multi-target drug molecules. These network pharmacology helps us to understand the molecular mechanism of Chinese traditional medicine in treatment diseases. Traditional Chinese Medicine Systems Pharmacology (TCMSP) system has provided a pharmacology framework for Chinese traditional medicine, and it is beneficial to study the molecular mechanism of drug action[10]. Our study used the bioinformatics method by data-mining of TCMSP, investigated the molecular mechanism of tataricus L. f. in treating radiation pneumonitis. As a result, we expected to provide an idea for the follow-up study on the mechanism of feining granule.

Materials and Methods

The main process of this study is shown in the figure (Figure 1). By searching for the main targets related to aster tataricus L. f. and the main targets of radiation pneumonia, the overlap genes were got and the subsequent analysis was carried out.

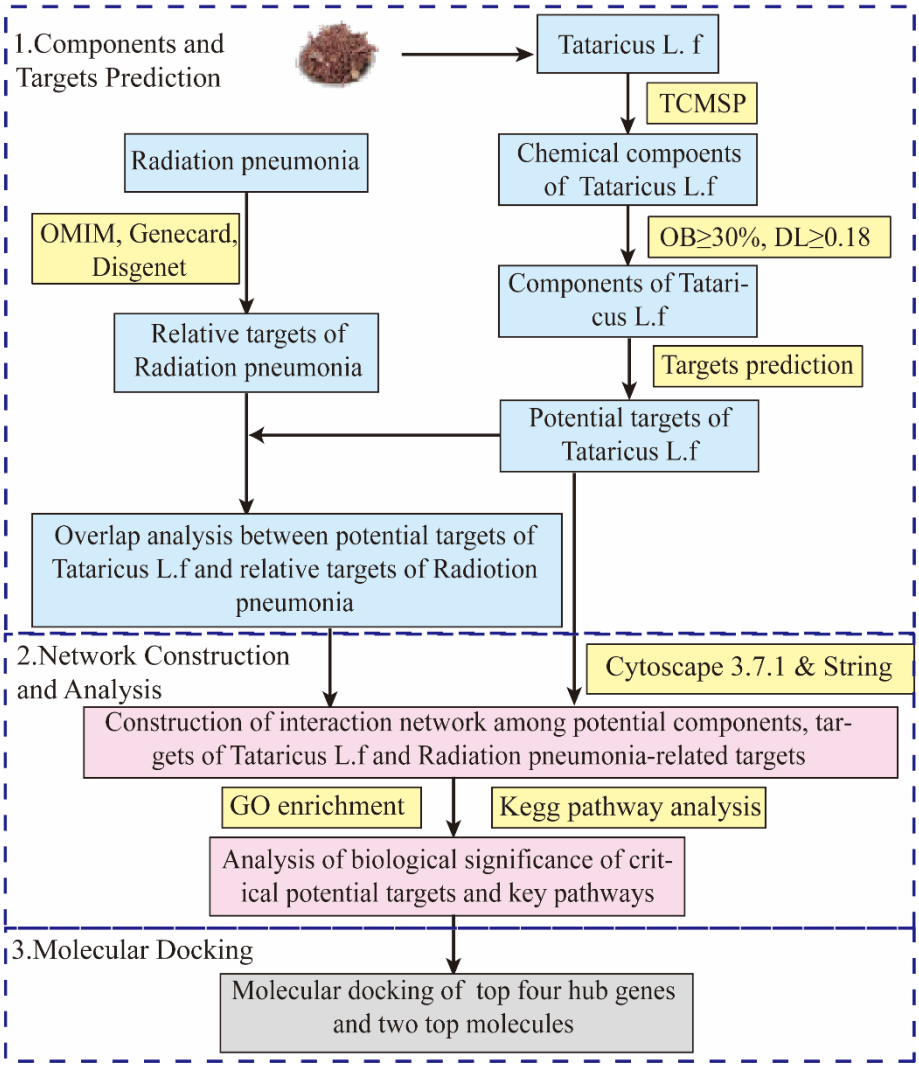

Figure 1 The process of this study

Data preparation-to obtain ingredients and target genes

Sign up for and log on to the Traditional Chinese Medicine Systems Pharmacology (TCMSP) website (https://tcmsp-e.com/). Search in the website with the keywords "Ziwan", and we found ingredients and target genes. Use the filter provided on the site to select ingredients with Oral bioavailability (OB) greater than or equal to 30% and drug-likeness (DL) greater than or equal to 0.18%. Then, the corresponding target genes were found, according to the selected ingredients. We transformed the targeted gene names to gene symbols through the website of Uniprot (https://www.uniprot.org).

Acquire the genes associated with radiation pneumonitis

Using "Radiation pneumonitis" or "Radiation-induced lung injury" as the key word, we searched the databases of Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), Genecard (https://www.genecards.org/), Disgenet (https://www.disgenet.org/home/) for genes associated with "Radiation pneumonitis".

Network construction and Protein–protein interaction analysis

To construct the network of interactions, we cross-referenced genes associated with diseases and drugs. We have created networks of diseases, drugs, and genes by

using Cytoscape 3.7.1 software, and drew Protein-protein interaction network through Sting website (https://string-db.org/) and Cytoscape software. Set “hightest confidence (0.9)” for the filter condition “Minimum required interaction score” in the String website. R software were used to sifting for the top 30 genes by analyzing the strength of interaction.

Go and Kegg analysis for genes

For GO (Gene Ontology) and Kegg (Kyoto Encyclopedia of Genes and Genomes) pathway analysis, we used the “stringi” and “cluster profiler” packages of R software. We selected twenty gene enrichment pathways by sorting the scores of every pathway.

Molecular docking experiments

Four key genes and two key drug molecules were screened from the networks of diseases, drugs, and genes. Download gene-encoded protein structures from the PDB website (https://www.rcsb.org/search/mypdb). The molecular components of traditional Chinese medicine were downloaded from the TCMSP website. We used the software of PyMOL(TM) 2.5.2 for protein and molecular structure transformation (including removal of water and residues, addition of hydrogen atoms, etc.). The software of Vina.exe was used to achieve molecular docking. Finally, we used PyMOL(TM) 2.5.2 software to visualize the results of molecular docking.

Result

Identification of Active Compounds and targets in aster tataricus L. f.

From the TCMSP, 19 major molecules of aster tataricus L. f. were selected. A total of 384 targets were identified for 13 of those molecules. The other 6 molecules have no known targets. Subsequently, we carried out to reprocess, and obtained the gene name through the website of Uniprot. Eliminated duplicate gene names, a total of 193 target genes were obtained.

Identification and acquisition of Radiation Pneumonitis-related genes

Used the keywords “Radiation pneumonitis” or “Radiation-induced lung injury” to search the databases of OMIM, Genecard, and Disgenet websites, and found 206 genes, 1,823 genes and 4 genes, respectively. By eliminating duplicates, 897 genes were obtained. Finally, we obtained 111 genes by crossing drug and disease related target genes (Figure 2).

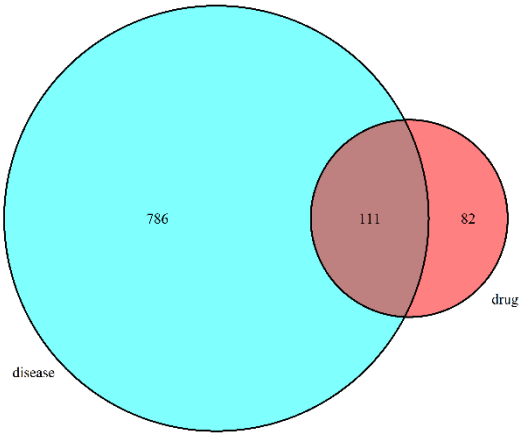

Figure 2 The intersection of drug-and disease-related genes.

Construction of gene interaction network

To make it easier to show which genes are involved in the treatment of disease, we mapped the network by using Cytoscape software. A total of 111 genes and 10 drug components were used to map the network. We found that quercetin, luteolin and kaempferol, as the main components, play a key role in the treatment of radiation pneumonitis by aster tataricus L. f. By sorting the numerical value of degree, we found that the regulation of PTGS2, PIK3CG, PPARG and HSP90AA1 genes plays an important role in the treatment of radiation pneumonitis (Figure 3 A). To further explore the key genes, the protein-protein interaction network was mapped online via the Sting website (Figure 3 B). Under the condition that the computer prediction is highly interactive, counting the number of genes that interact, we identified the first 20 genes that play an important role in regulating the entire gene network (Figure 3 C).

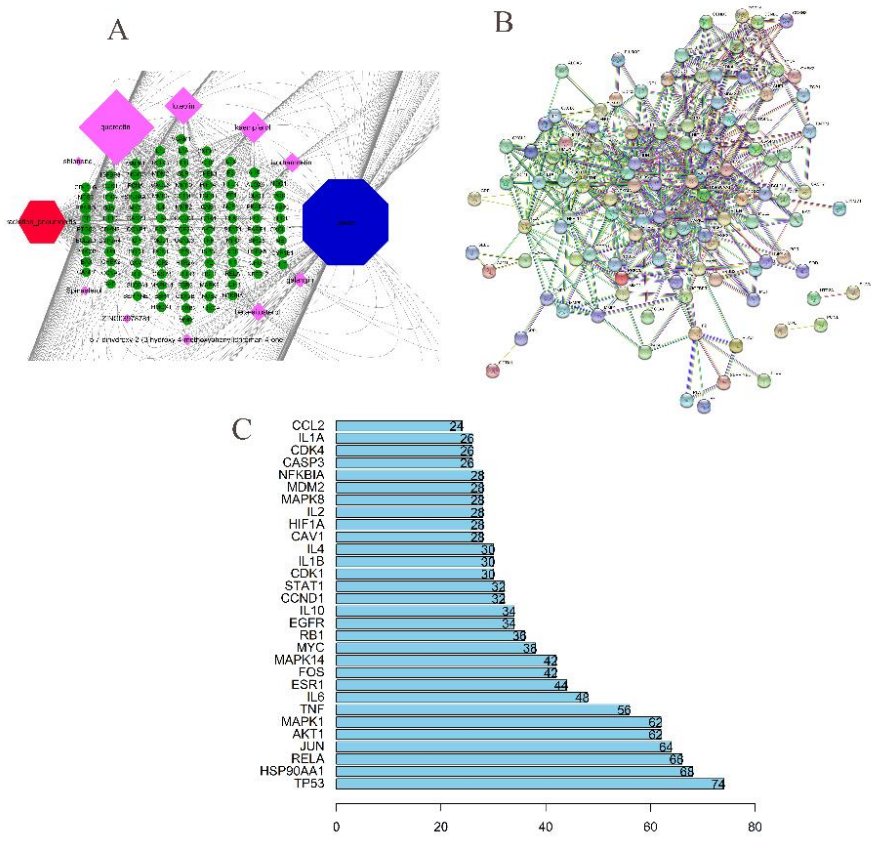

Figure 3 Network construction and Protein–protein interaction analysis.

A The networks of diseases, drugs, and genes.

B Mapping the protein-protein interaction network

C The 20 Key genes in PPI network.

The result of Go and Kegg analysis

In order to better understand the function of these genes, we carried out gene function annotation analysis and pathway enrichment analysis. From the GO analysis, we observed that 20 of the 111 genes were enriched in the GO entry of DNA-binding transcription factor, which were some proteins required for the regulation of RNA polymerase by specific regulatory sequences in or near a gene. And 18 genes were enriched in the entry of RNA polymerase II-specific DNA-binding transcription factor and protein serine/threonine kinase activity. It is suggested that aster tataricus L. f may regulate the variable splicing of genes and the transcription of DNA in the treatment of radiation pneumonitis. Besides, aster tataricus L. f may regulate the process of ATP function by regulating the activity of protein Serine/threonine Kinase in treating radiation pneumonitis (Figure 4 A and B). After completing the Pathway analysis, we identified 166 critical pathways that are enriched. Lipid and arteriosclerosis are the most abundant pathways for gene enrichment, suggesting that radiation pneumonitis is associated with chronic inflammation induced by radiation therapy and that lung capillary plays an important role. In addition, asters can treat radiation pneumonitis by blocking this signal pathway (Figure 4 C, D, E, F, G, H, I).

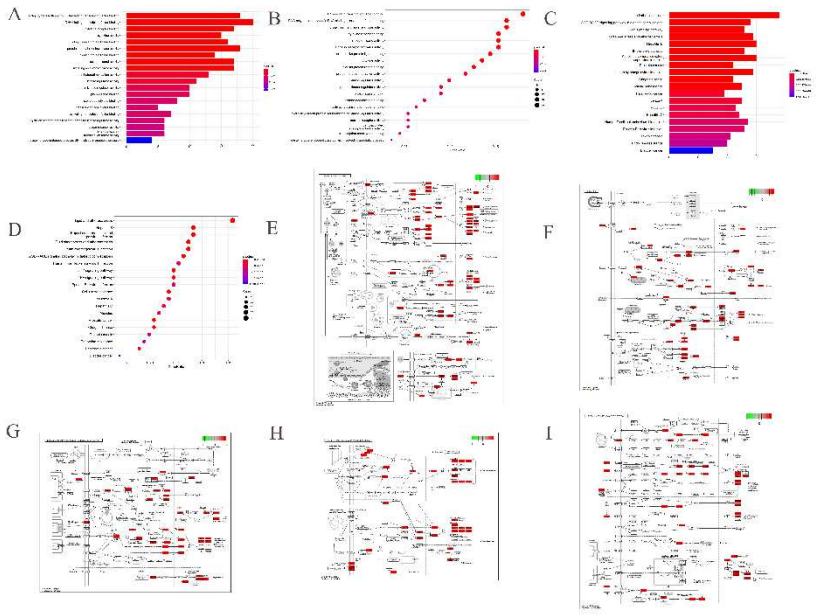

Figure 4 The result of Go and Kegg analysis.
A and B The result of Gene Ontology.
C, D, E, F, G, H, I The result of Kegg pathway analysis.

Molecular docking

The two top molecules in aster tataricus L. f and the proteins encoded by the top four hub genes in the PPI network were selected for molecular docking. A total of 8 pairs of molecular docking results are shown in the table. Set the binding energy threshold to -5 kcal/mol. Among the molecular docking results, we can see that the best binding molecules and proteins are luteolin binding to the protein encoded by *RELA* and *JUN* genes (both best affinity = -8.4 kcal/mol). The most tightly bound protein molecules with quercetin are the proteins encoded by the *JUN* and *RELA* genes (both best affinity = -8.3 kcal/mol) (Figure 5).

Results of molecular docking

Molecule	PDB	Gene	Best affinity
quercetin	1A1U	*TP53*	-5.9
quercetin	1BYQ	*HSP90AA1*	-7.6
quercetin	1NFI	*RELA*	-8.3
quercetin	1A02	*JUN*	-8.3
luteolin	1A1U	*TP53*	-6.3
luteolin	1BYQ	*HSP90AA1*	-7.6
luteolin	1NFI	*RELA*	-8.4
luteolin	1A02	*JUN*	-8.4

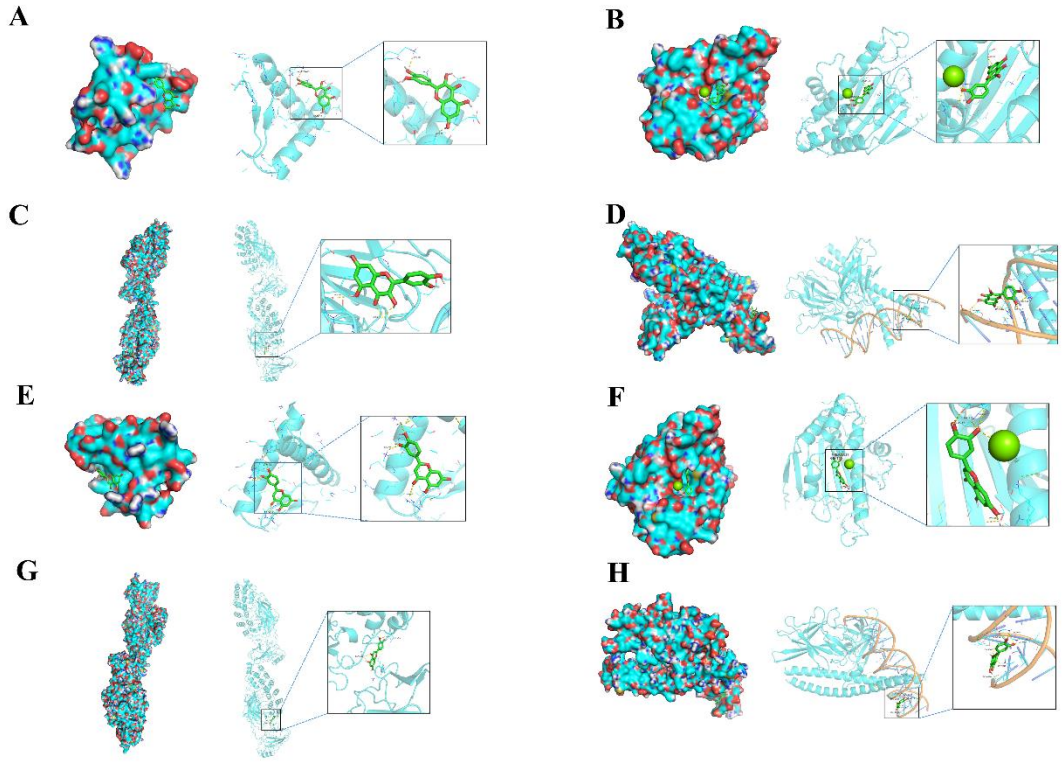

Figure 5 Molecular docking of bioactive molecules and hub genes.
A. quercetin to *TP53*, best affinity = -5.9 kcal/mol.
B. quercetin to *HSP90AA1*, best affinity = -7.6 kcal/mol.
C. quercetin to *RELA*, best affinity = -8.3 kcal/mol.
D. quercetin to *JUN*, best affinity = -8.3 kcal/mol.
E. luteolin to *TP53*, best affinity = -6.3 kcal/mol.
F. luteolin to *HSP90AA1*, best affinity = -7.6 kcal/mol.
G. luteolin to *RELA*, best affinity = -8.4 kcal/mol.
H. luteolin to *JUN*, best affinity = -8.4 kcal/mol.

Discussion

As one of the adverse reactions of radiotherapy for lung cancer, radiation pneumonitis has a high incidence and mortality rate. So far there are no effective drugs for prevention and treatment. In previous animal experiments, we observed that feining granule has a certain therapeutic effect on radiation pneumonitis. The preventive effect on radiation pneumonitis was better than the therapeutic effect, and its preventive and therapeutic effects may be related to the decrease of TGF-β, IL-1 and IL-6 levels[9]. In this study, through the network pharmacological analysis of aster tataricus L. f., which is the main component of feining granules, the possible target genes were identified. We have confirmed that TGF-β, IL-1 and IL-6 were important targets, in addition, more important targets have also been identified (Supplementary material).

TP53 is a tumor suppressor gene with high mutation probability in many solid tumors. It has also been shown to be an important target in acute lung injury models. Inhibiting TP53-mediated endogenous apoptotic pathway can reduce the inflammatory response in acute lung injury [11]. Moreover, pulmonary fibrosis with lung cancer is

not uncommon in the clinical real world. As literature suggests that some genes, including the TP53 gene, may be closely related to lung cancer with pulmonary fibrosis[12]. The main manifestation of chronic radiation pneumonitis is pulmonary fibrosis[13]. Therefore, TP53-targeted inhibitors may not only have therapeutic effects on tumor, but also become an important method for the treatment of radiation-induced pulmonary fibrosis.

In this predictive model, we also found associated inflammatory factors, such as, IL-6, IL-10, IL-1B, IL-2, IL-1A, TNF. Before our study, many studies have shown the mechanism of radiation pneumonitis. It has been reported that radiation-induced lung injury may activate genes NOX2, NOX4, DOUX1 and DOUX2, which lead to the increasing expression of ROS, and promoting phosphorylation of NF-KB and up-regulating inflammatory factors, such as, TNF-α, IL-6, IL-1β, and TGF-β1[14]. This strongly supports the results of our research. Additionally, when we analysis the effective components of aster tataricus L. f, we found that quercetin can effectively target these inflammatory factors and exert anti-inflammatory effects. This implies that quercetin may be a candidate natural medicine for the treatment of radiation pneumonia.

More importantly, pathway enrichment analysis helps us to discover the key signaling pathway of aster tataricus L. f in treatment of radiation pneumonia. Reviewing the previously published literature, some signaling pathways of radiation pneumonitis have been reported, such as, TGF-β1/Smad3 pathway[15], PI3K-Akt, HIF-1, TNF signaling pathways[16], and Inflammatory factor-related signaling pathways[17-19]. Through our analysis, we confirmed previous research, besides, we also found that the interaction of lipids and atherosclerosis, advanced glycation end products and their receptors, and interleukin 17 play an important role in the occurrence and development of radiation pneumonia, and those were also the key pathways for aster tataricus L. f. to exert its drug effect. In the long-term work, we will start with the discovered targets, signal pathways and active ingredients of the drug, and further verify the network analysis results to find more effective and safer drugs for the treatment of radiation pneumonia. The results of the molecular docking experiments gave us enough confidence to deduce that quercetin and luteolin are candidate small molecules for the treatment of radiation pneumonitis.

Although this study has discovered many targets and pathways, and analyzed the main drug components of aster, there are still many shortcomings in our study. Firstly, traditional Chinese medicine prescriptions are mostly compound preparations, and their medicinal effects often depend on the compound components of the prescriptions, and a single ingredient may not be able to exert its therapeutic effect. Secondly, network pharmacology can only predict the corresponding targets and pathways from a molecular perspective. To obtain reliable results, further verification by basic and clinical trials is required. Finally, due to the limitations of the database itself, the included data cannot be omitted without omission.

Conclusion

Through the pharmacological network analysis, we have obtained the key targets of aster tataricus L. f in the treatment of radiation pneumonia. Among these key targets, TP53, HSP90AA1, RELA, JUN, AKT1, MAPK1, TNF and IL-6 are the most critical

targets. This research result strongly confirms previous research[9]. These key targets are more focused on the gene ontologies of DNA-binding transcription factor, RNA polymerase II-specific DNA-binding transcription factor and protein serine/threonine kinase activity, and the pathways of lipids and atherosclerosis, advanced glycation end products and their receptors, and interleukin 17. This study reveals the mechanism of action of aster in the treatment of radiation pneumonia, but further basic and clinical studies need to be carried out to confirm the effectiveness and safety of its treatment.

Author Contributions

MmZ is the main completer of the study; Gc L gave important guidance in the process of data analysis; Hq Hu provided valuable information on the use of string website and the use of Cytoscape software; MY contributes to the effect of Feining granules in a rat model of radiation pneumonitis; Y L provided help in the installation of R language and the learning of basic knowledge; Jh L, Gm X, and Y T helped with the beautification and puzzle of the figure in the article; Z Y and X L gave guidance on the overall design of the article.

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