Quercetin and Luteolin may be the New Effective Drugs for Radiation Pneumonitis: Based on a Systems Pharmacology

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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 a feining granule is a potentially effective drug for the treatment of radiation pneumonitis, but its mechanism and main components are still unclear. Our study used bioinformatics methods to analyze the main drug Aster tataricus L. f. in feining granules and aims to gain the main mechanism in the treatment of radiation pneumonitis. Methods: Analyzed the effective drug components and targets of Aster tataricus through the Traditional Chinese Medicine Systems Pharmacology website. And obtained gene targets related to radiation pneumonia through the website of OMIM, Genecard, and Disgenet. Protein–protein interaction (PPI), gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (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: In total, 193 targets were identified for 13 molecules of Aster tataricus, and 897 genes were identified to be related to radiation pneumonia. Finally, we obtained 111 genes by crossing drug and disease-related target genes. Using PPI, GO, and KEGG analysis, we found TP53, HSP90AA1, RELA, JUN, AKT1, mitogen-activated protein kinase 1 (MAPK1), tumor necrosis factor (TNF), and interleukin-6 (IL-6) are the most critical genes, which were mainly focused on the GOs 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 IL-17. Conclusion: Through molecular docking experiments, it was found that the small molecules of quercetin and luteolin bind tightly to RELA and JUN proteins. We reveal the mechanism of action of Aster tataricus in the treatment of radiation pneumonia. Quercetin and luteolin may be effective small molecules for radiation pneumonitis.

Keywords
lungen cancer, radiation pneumonitis, Aster tataricus L. f., network pharmacology, traditional Chinese medicine

Introduction

Lung cancer is one of the malignant tumors with the highest mortality rate in the world, and its incidence remains high.1 Radiotherapy, as one of the main treatments of lung cancer, is a very important method for inoperable patients, especially for locally advanced and partially advanced lung cancer patients.2–4 However, the occurrence of radiation pneumonitis after radiotherapy often causes great suffering to patients. The incidence of radiation pneumonitis is as high as 30%, and nearly 20% of these patients require oxygen and other ventilation support. About 2% of these patients will die.5 This condition is more common in combination therapy, such as radiotherapy combined with chemotherapy, immunotherapy and targeted therapy and so on.6,7 Combination regimens are confirmed to be effective treatments for improving the
prognosis of lung cancer. 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 has achieved many good outcomes in the treatment of many diseases, such as Artemisinin for malaria, Tripterygium wilfordii for rheumatoid arthritis, etc. Feining granule is a traditional Chinese medicine patent prescription. It is used to treat lung infections, 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. 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, 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 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 systems and selects specific signal nodes (Nodes) to design multitarget drug molecules. This network pharmacology helps us to understand the molecular mechanism of Chinese traditional medicine in the treatment of diseases. A 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. In this study, bioinformatics methods were used to explore the molecular mechanism of tataricus in the treatment of radiation pneumonitis through data mining of TCMSP. As a result, we expected

Figure 1. The process of this study.
to provide an idea for the follow-up study on the mechanism of feining granules.

**Materials and Methods**

The main process of this study is shown in Figure 1. By searching for the main targets related to *A tataricus* and the main targets of radiation pneumonitis, the overlap genes were obtained and the subsequent analysis was carried out.

**Data Preparation to Obtain Ingredients and Target Genes**

Register and log into the TCMSP website (https://tcmsp-e.com/). Searching the website with the keywords “Ziwan” (the website search entry supports Chinese Pinyin name and Latin name, and “Ziwan” is the Chinese pinyin name of *A tataricus*), we found ingredients and target genes. Use the filters provided on the website to select ingredients with oral bioavailability ≥30% and drug-likeness ≥0.18%. Then, the according target genes were found. We converted 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 keywords, we searched the databases of Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), Genecard (https://www.genecards.org/), and 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 (PPI) network through the Sting website (https://string-db.org/) and Cytoscape software. Set “highest confidence (0.9)” for the filter condition “minimum required interaction score” on the String website. R software was used to sift for the top 30 genes by analyzing the strength of interaction.

**Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Analysis for Genes**

For gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, we used the “stringi” and “cluster profiler” packages of R software. We selected 20 gene enrichment pathways by sorting the scores of every pathway.

**Molecular Docking Experiments**

Four key genes and 2 key drug molecules were screened from the networks of diseases, drugs, and genes. Download gene-encoded

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**Figure 2.** The intersection of drug- and disease-related genes.
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™ 2.5.2 for protein and molecular structure transformation (including removal of water and residues, addition of hydrogen atoms, etc). The software “Vina.exe” was used to achieve molecular docking. Finally, we used PyMOL™ 2.5.2 software to visualize the results of molecular docking.

**Result**

*Identification of Active Compounds and Targets in A tataricus*

From the TCMSP, 19 major molecules of *A tataricus* 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 reprocessing and obtained the gene names through the website of Uniprot. Eliminated duplicate gene names, a total of 193 target genes were obtained (Supplemental Table 1).

*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, 1823 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 (Supplemental Table 2, Figure 2).

*Construction of Gene Interaction Network*

To more easily show which genes are involved in disease treatment, 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 *A tataricus*. By sorting the numerical value of the degree, we found that the regulation of prostaglandin-endoperoxide synthase 2 (PTGS2), phosphatidylinositol 3-kinase, catalytic subunit gamma (PIK3CG), peroxisome proliferative activated receptor gamma (PPARγ), and heat shock protein 90 alpha family class A member 1 (HSP90AA1) genes affects the efficacy of radiation pneumonitis (Supplemental Table 3, Figure 3A). To further explore the key genes, the PPI network was mapped online via the Sting website (Supplemental Table 3, Figure 3B). 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 (Supplemental Table 3, Figure 3C).

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 factors, 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 *A tataricus* may regulate the variable splicing of genes and the transcription of DNA in the treatment of radiation pneumonitis. In addition, *A tataricus* may regulate the process of adenosine triphosphate (ATP) function by regulating the activity of protein serine/threonine kinase in treating radiation pneumonitis (Supplemental Table 4, Figure 4A 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. We also found that *A tataricus* can treat radiation pneumonitis by blocking this signal pathway (Supplemental Table 4, Figure 4C-I).

Molecular Docking
The 2 top molecules in *A tataricus* and the proteins encoded by the top 4 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 $-5 \text{ kcal/mol}$. 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 $= -8.4 \text{ kcal/mol}$). The most tightly bound protein molecules with quercetin are the proteins encoded by the JUN and RELA genes (both best affinity $= -8.3 \text{ kcal/mol}$) (Figure 5 and Table 1).

Discussion
As one of the adverse reactions of radiotherapy for lung cancer, radiation pneumonitis has a high incidence and

![Figure 4](image.png)

**Figure 4.** The result of GO and KEGG analysis. (A, B) The result of GO. (C-I) The result of KEGG pathway analysis. Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
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 transforming growth factor-β (TGF-β), interleukin (IL)-1, and IL-6 levels. In this study, through the network pharmacological analysis of *A. tataricus*, which is the main component of feining granules, the possible target genes were identified. We have identified TGF-β, IL-1, and IL-6 as important targets, in addition, more important targets have also been identified (Supplemental material).

*TP53* is a tumor suppressor gene with a high mutation probability in many solid tumors. It has also been shown to be an important target in acute lung injury models. Inhibiting the *TP53*-mediated endogenous apoptotic pathway can reduce the inflammatory response in acute lung injury. Moreover, pulmonary fibrosis with lung cancer is not uncommon in the clinical real world. The relevant literature proves that some genes including the *TP53* gene may be closely related to pulmonary fibrosis and lung cancer. The main manifestation of chronic radiation pneumonitis is pulmonary fibrosis. Therefore, *TP53*-targeted inhibitors may not only have therapeutic effects on a 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-1β, IL-2, IL-1A, and tumor necrosis factor (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 increased expression of reactive oxygen species, and promote phosphorylation of nuclear factor kappa B (NF-KB) and up-regulating inflammatory factors, such as TNF-α, IL-6, IL-1β, and TGF-β1. This strongly supports the results of our research. Additionally, when we analyzed the effective components of *A. tataricus*, we found that quercetin can effectively target these inflammatory factors and exert anti-inflammatory effects. It has been previously reported that quercetin can block the secretion of IL-1β, IL-6, interferon-γ, and TNF-α induced by lipopolysaccharide, and inhibit proinflammatory cytokines. This implies

![Molecular docking of bioactive molecules and hub genes.](image)
that quercetin may be a candidate natural medicine for the treatment of radiation pneumonitis.

More importantly, pathway enrichment analysis helps us to discover the key signaling pathway of *A tataricus* in the treatment of radiation pneumonitis. Reviewing the previously published literature, some signaling pathways of radiation pneumonitis have been reported, such as the TGF-β1/Smad3 pathway,20 phosphatidylinositol 3-kinase-Protein kinase B (PI3K-Akt), hypoxia-inducible factor-1 (HIF-1), and TNF signaling pathways,21 and inflammatory factor-related signaling pathways.22–24 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 IL-17 play an important role in the occurrence and development of radiation pneumonitis, and those were also the key pathways for *A tataricus* 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 pneumonitis. 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 *A tataricus*, there are still many shortcomings in our study. First of all, traditional Chinese medicine prescriptions are mostly compound preparations, and their medicinal effects often depend on the compound components of the prescriptions, and a single component 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 *A tataricus* in the treatment of radiation pneumonitis. Among these key targets, TP53, HSP90-AA1, RELA, JUN, AKT1, mitogen-activated protein kinase-1 (MAPK1), TNF, and IL-6 are the most critical targets. This research result strongly confirms previous research.13 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 IL-17. This study reveals the mechanism of action of *A tataricus* in the treatment of radiation pneumonitis, but further basic and clinical studies need to be carried out to confirm the effectiveness and safety of its treatment.

**Author Contributions**

MZ is the main completer of the study; GL gave important guidance in the process of data analysis; HH provided valuable information on the use of string websites and the use of Cytoscape software; MY contributed to the effect of Feining granules in a rat model of radiation pneumonitis; YL provided help in the installation of R language and the learning of basic knowledge; JL, GX, and YT helped with the beautification and puzzle of the figure in the article; ZY and XL gave guidance on the overall design of the article.

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**Declaration of Conflicting Interests**

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**Ethical Approval**

Ethical Approval is not applicable for this article.

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**Statement of Human and Animal Rights**

This article does not contain any studies with human or animal subjects.

**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.

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**Table 1. Results of Molecular Docking**

| Molecule   | PDB     | Gene   | Best affinity |
|------------|---------|--------|---------------|
| Quercetin  | 1A1U    | TP53   | −5.9          |
| Quercetin  | 1BYQ    | HSP90-AA1 | −7.6        |
| Quercetin  | 1NFI    | RELA   | −8.3          |
| Quercetin  | 1A02    | JUN    | −8.3          |
| Luteolin   | 1A1U    | TP53   | −6.3          |
| Luteolin   | 1BYQ    | HSP90-AA1 | −7.6        |
| Luteolin   | 1NFI    | RELA   | −8.4          |
| Luteolin   | 1A02    | JUN    | −8.4          |
Supplemental Material

Supplemental material for this article is available online.

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