

Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-23-1492>

Reviewer A

The authors have studied the potential of traditional Chinese medicine for treating ground glass nodules (GCNs). Network pharmacology modelling was developed to identify the active ingredients and their targets of three TCM prescriptions. The biological processes of the mechanisms of action were predicted from the proteomics analysis. Furthermore, the compound called quercetin was discovered by docking.

Major comments:

Comment 1: In the literature review, similar approaches involving network modelling to discover traditional Chinese medicine should be surveyed, such as: <https://pubmed.ncbi.nlm.nih.gov/33834186/>; <https://pubmed.ncbi.nlm.nih.gov/32982738/>.

Reply 1: I am grateful for your suggestions on modifying the introduction of the manuscript. Network pharmacology is a diverse field of research that uses computational analysis, in vivo and in vitro experiments, and extensive information to identify new drug targets and molecular mechanisms. It combines systems biology, genomics, proteomics, and other disciplines to achieve this objective [1]. The methods utilized in network pharmacology include calculating network topology information, creating and comparing random networks, layering, and clustering, and visualizing networks. Experimental techniques involve high-throughput omics methods, in vivo and in vitro biology, and pharmacology experiments.

The origins of network pharmacology can be traced back to the study of traditional Chinese medicine (TCM) and has developed in parallel with it. Network pharmacology predicts disease genes, drug targets, and drug functions and constructs and analyzes Chinese herbal medicine networks and drug-gene-disease networks [2]. It is a unique system based on traditional medical knowledge and differs fundamentally from the modern medicine approach of the "drug-target-disease" model. The TCM approach emphasizes "diagnosis and treatment" and "holistic perspective" in addressing illnesses, providing a natural framework for exploring combination therapy. TCM formulas are developed by combining Chinese herbs in compounded preparations, following "the seven methods in prescription compatibility" and "Jun Chen Zuo Shi" to achieve a comprehensive treatment approach [3]. In recent years, there has been a growing international recognition of TCM and a need to modernize it through scientific research. However, the complexity of TCM components and their interactions with biological systems make it challenging to understand their molecular mechanisms. The lack of clarity on the effective substances in Chinese herbal formulas is a significant obstacle in TCM research. Network pharmacology offers a way to identify potentially effective components and map them to disease gene networks to find pharmacodynamic

substances. It is crucial to uncover the molecular mechanisms and bioactive markers of TCM and its toxic effects to standardize TCM treatments. TCM treatment involves multiple compounds and targets, and network pharmacology systematically analyzes multi-target and multi-pathway pharmacological effects. Therefore, network pharmacology has become a valuable tool for studying the therapeutic mechanisms of TCM and has helped to decipher the scientific basis of TCM. Significant progress has been made in understanding the essential characteristics of TCM and discovering its multiple effects on various pathways, targets, and components through network pharmacology. Network pharmacology and TCM share the same core idea and complement each other in advancing research in this field.

This study will uncover the potential bioactive substances found in Chinese herbal formulas for treating ground glass nodules (GGNs) by searching various databases. It is based on previous research that uses network modeling to simulate traditional Chinese medicine [2, 4]. It provides new insights into the rationale behind herb classifications and advances the exploration of traditional Chinese medicine pharmacology for GGNs. We have added the details of previous studies which similar to our study design in Introduction section.

Changes in the text: page 5, line 120 to 123.

Comment 2: It is unclear how the hub genes were determined. On page 6 line 137 it says ‘five hub genes’ but it does not specify what metric is used to determine the centrality of the genes in the network.

Reply 2: We thank the reviewer for their insightful observation and for revising the manuscript to provide a more explicit explanation regarding the metric used for determining the centrality of genes. The results were visualized using Cytoscape software after constructing the protein-protein interaction (PPI) network utilizing the STRING website. The PPI network comprises 13 nodes, including ALOX5, MPO, ICAM1, PPARG, VEGFA, TNF, CD40LG, IFNG, CRP, STAT3, THBD, EGFR, and MAPK3, with a total of 61 interacting pairs. To identify central genes within the network, we utilized the Maximum Clique Centrality (MCC) measure in the Cytohubba plug-in in Cytoscape. The MCC measure is crucial for identifying genes that participate in the largest clique within the network and play a significant role in key biological processes leading to disease [5]. Our analysis identified the top 5 nodes with the highest MCC connection scores as central genes forming one subnetwork in our study. In the methods section, we have added a description of how the hub gene was determined. We have also supplemented the central gene's MCC score in Table S4 and added description in the Result section.

Changes in the text: page 7 and 11, line 163 to 171, 267 to 274 and Table S4.

Comment 3: Limitations of the current study include the lack of experimental validation on the drug targets, such as functional screening such as knockout or drug screening, outlined in <https://pubmed.ncbi.nlm.nih.gov/37485338/>. These limitations should be discussed.

Reply 3: Thanks for your kind reminder. Through network pharmacology, this study

explored potential targets and mechanisms for TCM prescriptions in treating GGNs and identified an effective ingredient in TCM prescriptions. Nevertheless, the lack of experimental data support may diminish the persuasiveness of our viewpoint, which is one of the limitations of this study. Several studies have demonstrated modeling and methods such as drug sensitivity analysis, cell culture, transfection, quantitative real-time PCR, etc. [6-9] to predict drug properties. Building on the findings of this study, our future research will draw from these previous studies to investigate whether this effective ingredient could be utilized as a new treatment. We have added a description of limitation in the Discussion section.

Changes in the text: page 14, line 361 to 363.

Minor comments:

Comment 4: Extra space is needed before the citation brackets ().

Reply 4: I am thankful for your revised recommendation regarding the format of citation. We have modified and ensured that the citation format of the entire text meets the requirements.

Changes in the text: All citations in the manuscript.

Comment 5: Wayne diagram -> Venn diagram

Reply 5: Thanks for your reminder. we have replaced the “Wayne diagram” with “Venn diagram”.

Changes in the text: page 6, line 158.

Comment 6: Fonts in Figure 2 and Figure 4 are generally too small. Suggest to increase the sizes to make them more readable.

Reply 6: Thanks for reminding us of the size of fonts from Figure 2 and Figure 4, we have rescaled the typography and resolution of the Figure 2 and Figure 4.

Changes in the text: Figure 2 and Figure 4.

Comment 7: Resolution of Figure 5 needs to be improved.

Reply 7: Thanks for reminding us of the resolution from Figure 5, we have rescaled the typography and resolution of the Figure 5.

Changes in the text: Figure 5.

Reviewer B

This article investigates three Chinese herbal formulations in treating lung adenocarcinoma by targeting ground glass nodules. All the results are based on network pharmacology and molecular docking.

Several recommendations are made below for the suggested changes before the acceptance of the paper:

Comment 1: The introduction needs to be elaborated on the background information of the selected three prescriptions, if they have been used against lung adenocarcinoma

or related symptoms in clinics, and if there is traditional knowledge.

Reply 1: Thank you for reminding me of the lack of description in three traditional Chinese medicine prescriptions. Our research has revealed the utilization of three traditional Chinese medicine formulas, Chai Hu Shu Gan San (CHSGS), Li Shi Jian Zhi Shu Fang (LSJZSF), and Si Ni San He Sheng Jiang San (SNSHSJS), in the treatment of GGNs by analyzing a wide range of literature from various databases. Traditional Chinese medicine often relies on knowledge passed down from esteemed experts through inheritance studios, without further validation of their treatment methods and published articles. This means that relying solely on existing Chinese and English databases to search for traditional Chinese medicine treatments may overlook valuable prescriptions. During our database search, we came across a treatment proposed by the well-known Chinese medicine expert Li Kongding, which is commonly used for treating interstitial pneumonia but not referenced in many other articles. Additionally, previous research has also demonstrated the use of CHSGS and SNSHSJS in cancer treatment [3, 10-12]. Furthermore, the primary ingredient in CHSGS, bupleurum, has been found to possess antioxidant, anticancer, and apoptotic properties [13, 14]. We have added the details of three prescriptions in the Induction section.

Changes in the text: page 5, line 114 to 120.

Comment 2: Why quercetin is particularly highlighted? The rationale needs to be explained in the introduction, results or discussions.

Reply 2: Thanks for your useful comment. GGNs in the lung pose a significant risk for lung adenocarcinoma, and immediate surgery is not recommended for patients with GGNs. Instead, patients are often monitored using low-dose CT scans, which can be emotionally and financially burdensome. As traditional Chinese medicine (TCM) becomes more widely accepted, this study aimed to investigate whether TCM could be beneficial for treating GGNs and the potential mechanisms behind it. We analyzed traditional Chinese medicine formulas in the database. We found that quercetin is the effective ingredient with the highest score in treating GGNs, based on the standard of Oral bioavailability $\geq 30\%$ and Drug-likeness ≥ 0.18 . In enrichment analysis, we also discovered that quercetin can impact the HIF-1 and MAPK signaling pathways to induce cancer cell apoptosis. Previous research has shown that quercetin can slow the progression of lung adenocarcinoma by reducing cell proliferation, migration, and invasion [15, 16]. Therefore, we believe that the significant impact of quercetin is a crucial factor in the effective treatment of GGNs using three Chinese herbal formulations. We have modified the description of the screening process of active ingredient in Results section and added the previous research results of quercetin in Discussion section.

Changes in the text: page 11, 13 and 14, line 272 to 274, 326 to 328 and 356 to 358.

Comment 3: In fact, the steps for screening major chemical compounds from the three prescriptions and if any compounds present high bioavailability and drug properties are missing.

Reply 3: We appreciate your feedback and have revised our manuscript to provide further clarity on the screening process. In our research, we used the Traditional Chinese Medicine Systems Pharmacology (TCMSP, <http://tcmsp-e.com>) platform to choose active components from each herbal medicine meticulously. TCMSP is a specialized platform for systems pharmacology of Chinese herbal medicines. It maps out the connections between drugs, targets, and diseases, and provides 12 pharmacokinetic properties, such as oral bioavailability (OB) [17], drug-likeness (DL), drug half-life, and blood-brain barrier permeability. OB indicates the percentage of an orally administered drug dose that reaches the systemic circulation, reflecting the absorption, distribution, metabolism, and excretion (ADME) process. DL is a qualitative measure used in drug design to assess how closely a potential compound resembles a typical drug, aiding in optimizing pharmacokinetic and pharmaceutical properties. [18]. In general, the selection of active components involves meeting specific criteria for oral bioavailability ($OB \geq 30\%$) and drug-likeness ($DL \geq 0.18$) to address concerns about bioavailability and drug properties. Our research specifically focused on identifying Chinese medicine ingredients in three different formulas (CHSGS, LSJZSF, and SNSHSJS) within the TCMSP database. We used the specified criteria to locate the sources listed in Table S1, Table S2, and Table S3. The identified ingredients consist of a variety of herbs, including Chaihu, Baishao, Ezhu, Danggui, Chuanxiong, Yujin, Xiangfu, Dihuang, Zhimu, Mihoutaogen, Baihuasheshecao, Shanzhuyu, Suanzao, Tusizi, Baiziren for CHSGS; Gancao, Zhike, Yuxingcao, Lianqiao, Huangqin, Huangjing, Nanshashen, Zhebeimu, Chishao for LSJZSF; and Chaihu, Baishao, Jiangcan, Jianghuang, Chantui, Zhike, Gancao for SNSHSJS. In the Methods section, we provide a more detailed description of the screening process.

Changes in the text: page 6, line 138 to 151.

Comment 4: The network of major components from three formulations - gene targets - pathways is missing.

Reply 4: Thank you for your reminder. We recognize this concern and have made efforts to tackle it. In our updated manuscript, we have provided a comprehensive illustration of the network, including major components, associated gene targets, and pathways, in Figure 4D.

Changes in the text: page 7 and 11, line 179 to 180 and 262 to 265, Figure 4 and its legends.

Comment 5: The major findings and the significance of the study is not highlighted.

Reply 5: Thanks for pointing out the lack of significance of the study's main findings in the Discussion section. Through the use of various databases and network pharmacology, we have identified the potential mechanisms of traditional Chinese medicine prescriptions for treating ground-glass nodules (GGNs). The Chinese medicines CHSGS, LSJZSF, and SNSHSJS, which are commonly used in clinical treatment of GGNs, all contain the common ingredient quercetin. This suggests that quercetin may be the primary element responsible for the therapeutic effects of these medicines. Our pathway enrichment analysis and molecular docking studies have

revealed that quercetin can target CRP, TNF, IFN- γ , ICAM1, and VEGFA by activating the HIF-1 signaling pathway and MAPK signaling pathway, leading to cancer cell apoptosis and exhibiting anti-tumor effects. Therefore, we propose that Chinese medicine formulas containing quercetin may be effective in treating GGNs. This research not only demonstrates the potential of Chinese medicines in treating pulmonary nodules, but also suggests that quercetin could be used as a new treatment formula for GGNs, warranting further study. These findings provide new insights for the clinical treatment of GGNs. We have added the main findings and significance of our research in the Discussion section and summarized the mechanism of quercetin in treating GGNs in the Conclusion section.

Changes in the text: page 14 and 15, line 356 to 358 and 365 to 370.

Comment 6: Following the molecular docking, the experimental validation should be followed.

Reply 6: Thank you for your kind advice, but due to the design of the experiment and the various factors considered, we were unable to verify our views through the experimental results after modeling and analysis in this study, which is one of the shortcomings of this article. In future research, we will strive to improve the therapeutic effect and mechanism of traditional Chinese medicine preparations in GGNs through experimental data.

Changes in the text: page 14, line 361 to 363.