Peer Review File

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Reviewer A

The manuscript by Zeng et al. discusses a computational approach to identify the targets of daucosterol that are relevant to multiple myeloma (MM). The authors overlapped the targets from approved drugs with those of daucosterol, and compared them to the MM gene set to identify potential targets for daucosterol. The authors further validated the identified targets via molecule docking.

Here are some comments on the paragraph

Comment 1: The list of approved drugs used for comparison is mostly composed of chemotherapeutics and is not specific to the treatment of MM. As a result, the drug list is biased and the targets identified are more related to proliferation, survival, checkpoints, etc. that are also targets for other types of cancer.

Reply 1: Dear reviewer, thank you for your very careful and meticulous review of the manuscript. The marketed drugs used for MM treatment were searched in the DrugBank database using the keyword "Multiple myeloma", and the list of drugs can be reproduced using this method.

Changes in the text: No changes in the text.

Comment 2: No approach was applied to identify targets specific to MM. MM is a malignancy of plasma cells, but most, if not all, of the targets listed are not relevant to plasma cells.

Reply 2: Dear reviewer, the question you asked is very professional. Perhaps it is because we used not only the data from the database, but also the data from the transcriptome to obtain MM related genes. This has led to the emergence of many new MM related genes, making some genes appear unfamiliar.

In the Method section of "**##Construction of specific gene set of MM**", We have established clear rules for obtaining specific gene set of MM. In this study, we aimed to systematically construct specific molecular network highly related to the pathogenesis of MM. In order to achieve this goal, we integrated the published scattered scientific research results, which were collected from DisGeNET, Open Target Platform, MalaCards, OMIM, GeneCards, and CTD. To ensure the credibility of MM gene set, we only retained genes that appeared in at least 4 data sources.

Changes in the text: No changes in the text.

Comment 3: It is unclear how the targets of daucosterol were identified. Were they identified from experiments or a database? Reference 24 describes a general method of identifying targets, but not specifically for daucosterol.

Reply 3: Dear reviewer, the question you asked is very professional. To improve reliability, the known targets and the putative targets were integrated to obtain the target profiles of the drugs. As for the known targets, they are identified from experiments, and the putative targets were predicted through multiple methods, such as, STITCH, SEA, TargetNet, SwissTargetPrediction, ChEMBL_prediction tool, and BATMAN-TCM.

Reference 24 is intended to illustrate that obtaining target data for drugs is a necessary basis for elucidating the mechanism of action.

Changes in the text: No changes in the text.

Comment 4: It is unclear whether each source of information was equally considered, or if weight was applied.

Reply 4: Dear reviewer, thank you for your very careful and meticulous review of the manuscript. We did not provide a detailed description of the threshold for each target prediction method. This is because we conducted the most rigorous threshold screening for the predicted source data before summarizing it, which is generally more stringent than the threshold reported in the literature. For example, STITCH and TargetNet, we use a threshold greater than 0.9.

We have established clear rules for obtaining target spectra: All the known targets were kept. Only the putative targets which could be predicted in at least 2 prediction models and validated by literature mining source at the same time were preserved. In the Method section of "**##Acquisition of potential target profiles of daucosterol and the approved drugs for** MM"

Changes in the text: No changes in the text.

Comment 5: The manuscript claimed that daucoserol may regulate the targets based on the binding data. However, protein-protein interaction does not mean regulation. Authors need to further investigate if the binding regions have any functions.

Reply 5: Dear reviewer, Dear reviewer, your suggestion is constructive. In order to enhance the reliability of the conclusions obtained from the data analysis in this study, multiple prediction methods were specifically used to obtain the target spectrum data of daucosterol. The target prediction algorithms used in this study are also based on diverse principles, and the predicted targets may not have affinity or may indirectly affect gene or protein expression changes. Affinity data is only a preliminary validation of individual key targets. Conducting in-depth functional research on the binding regions of key target proteins is also a fascinating research direction. We will

incorporate your suggestions when verifying the effectiveness of the target in subsequent experiments to investigate the functional significance of the binding regions. For example, the authors could conduct additional experiments to measure changes in gene expression or protein activity in response to daucosterol treatment, or perform structural analyses to identify how daucosterol binding affects the conformation or activity of the target proteins. By conducting additional experiments to investigate the functional significance of the binding regions, the authors can provide stronger evidence for the potential regulatory effects of daucosterol on its targets. This will help to strengthen the manuscript's overall argument and support the potential use of daucosterol as a treatment for multiple myeloma.

Changes in the text: No changes in the text.

Comment 6: The supplementary data is not available for review.

Reply 6: Thank you very much for your kind reminder. During the submission process, we submit supplementary data. For your convenience in reviewing and preventing data loss, we have resubmitted supplementary data.

Changes in the text: No changes in the text. we have modified our text as advised (see Page xx, line xx)"].

Reviewer B

The paper titled “Network pharmacology- and molecular docking-based investigation of the therapeutic potential and mechanism of daucosterol against multiple myeloma” is interesting. This study highlights the use of daucosterol as a promising therapeutic drug for MM treatment. These data provide new insights into the potential mechanism of daucosterol in the treatment of MM, which may provide references for subsequent research and even the clinical treatment. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: The pharmacodynamic indicators of daucosterol's regulating effect on multiple myeloma should be increased by experimental research. This is more conducive to support the conclusion of this paper.

Reply 1: Thank you very much for your suggestion. In the discussion section, we provided a detailed explanation of the research plan for subsequent experimental validation. This study focuses on exploring the therapeutic potential of daucosterol for MM from a computational perspective, providing reference for subsequent validation studies.

Conducting experimental research to evaluate the pharmacodynamic indicators of daucosterol's regulating effect on multiple myeloma would be important in supporting

the conclusion of this. By conducting experimental research to evaluate daucosterol's pharmacodynamic effects on multiple myeloma, researchers could gain a better understanding of how the compound interacts with cancer cells, how it affects the underlying biology of the disease, and what specific effects it has on tumor growth and progression. This information could help to provide stronger evidence for the potential effectiveness of daucosterol as a treatment for multiple myeloma and guide future clinical trials or drug development efforts.

Changes in the text: No changes in the text.

Comment 2: In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 2: The reviewer's comment is a common and important suggestion for manuscript improvement. In the introduction section of a manuscript, we have clearly state the knowledge gaps and limitations of prior studies, as well as the clinical significance of the current study.

Changes in the text: No changes in the text.

Comment 3: In addition to the drug in this study, what other drugs have more or less advantages in terms of effectiveness and acceptability in the treatment of multiple myeloma? What are the advantages of daucosterol? It is recommended to add relevant descriptions.

Reply 3: Multiple myeloma is a complex disease, and treatment options depend on factors such as disease stage, patient age, and overall health. There are several drugs that have been used in the treatment of multiple myeloma, each with their own advantages and disadvantages.

Some common drugs used in the treatment of multiple myeloma include:

a) Chemotherapy agents, such as melphalan and cyclophosphamide
b) Proteasome inhibitors, such as bortezomib, carfilzomib, and ixazomib
c) Immunomodulatory drugs, such as thalidomide, lenalidomide, and pomalidomide
d) Monoclonal antibodies, such as daratumumab and elotuzumab
e) Steroids, such as dexamethasone and prednisone

The advantages of each drug depend on factors such as the patient's response to treatment, side effects, and overall prognosis. For example, proteasome inhibitors are often effective in treating multiple myeloma, but they can also cause side effects such as peripheral neuropathy. Immunomodulatory drugs can be effective in treating multiple myeloma, but they can also increase the risk of blood clots.

Daucosterol is a phytosterol, which is a type of plant-derived compound that has been investigated for its potential anti-cancer properties. Some studies have suggested that daucosterol may have anti-tumor effects in multiple myeloma cells, as well as other

types of cancer cells. However, more research is needed to determine the efficacy and safety of daucosterol as a potential treatment for multiple myeloma.

Changes in the text: No changes in the text.

Comment 4: Figures 3 and 7 are not clear enough. It is recommended to provide clearer figures again.

Reply 4: Thank you very much for your kind reminder. We have resubmitted Figures 3 and 7 in the revised manuscript.

Changes in the text: No changes in the text. we have replaced Figures 3 and 7, Figure S2 with more clear versions (see Page 21 and 25)"].

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Study on the mechanism of Ginseng-Gegen for mesenteric lymphadenitis based on network pharmacology, Transl Pediatr, PMID: 36247894". It is recommended to quote the article.

Reply 5: We have quoted this article in the introduction part of this paper. We think the "22.Zhao S, Li S. A co-module approach for elucidating drug-disease associations and revealing their molecular basis. Bioinformatics 2012; 28:955-61." is not suitable and replaced it to "Study on the mechanism of Ginseng-Gegen for mesenteric lymphadenitis based on network pharmacology, Transl Pediatr, PMID: 36247894".

Changes in the text: No changes in the text. we have modified our text as advised (see Page 4, the third line of the last paragraph)"].

Comment 6: The latest application progress of network pharmacology in Chinese medicine research should be added to the discussion.

Reply 6: Thank you very much for your suggestion. In the introduction section, we explained the progress in the application of network pharmacology in traditional Chinese medicine research, and indirectly demonstrated that the application of this technology in this study is feasible. In the discussion section, we focused on a detailed discussion and analysis of the data analysis results. The author believes that there is no need to conduct extensive discussions or explanations on the application of network pharmacology technology in traditional Chinese medicine research.

Changes in the text: No changes in the text.

Comment 7: The functional research on the main target genes should be increased, which may be more meaningful.

Reply 7: Thank you very much for your suggestion. In the discussion section, we provided a detailed explanation of the research plan for subsequent experimental validation. This study focuses on exploring the therapeutic potential of daucosterol for

MM from a computational perspective, providing reference for subsequent validation studies.

Changes in the text: No changes in the text.