Peer Review File

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Reviewer A

The paper titled "Inhibition of TWF1 expression promotes lung adenocarcinoma progression and is associated with poor prognosis of cancer patients via the MMP1 signaling pathway" is interesting. TWF1 overexpression was correlated with poor prognoses and immune status of LUAD patients. Inhibited TWF1 expression delayed the growth and migration of cancer cells by downregulating MMP protein, implying that TWF1 is a promising biomarker for the prognoses of LUAD patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the relationship between TWF1 and tumor-infiltrating immune cells and the role of TWF1 play in prognosis in LUAD.

Reply: We have modified our text as advised (see Page 2, line 48-54).

2) Figures 6 is not clear enough. It is recommended to provide clearer figure again.

Reply: As images embedded in the manuscript may be compressed, resulting in reduced clarity, we have uploaded high-resolution TIFF format images alongside the manuscript for improved visual quality.

3) There are still some weak points in this paper. It is suggested that the author increase the research of signaling pathway. This is more conducive to support the conclusions of this study.

Reply: This suggestion is invaluable, and we have already begun exploring the related content in our next research project. Consequently, the relevant findings may be presented in the context of that subsequent study.

4) It is recommended to increase the study of lncRNA or miRNA to regulate the TWF1, which may make the whole study more complete.

Reply: Our next research project focuses on studying the long non-coding RNAs (lncRNAs) that can regulate TWF1 and the pathways they jointly modulate. Therefore, we may reveal the relevant findings in the context of this upcoming study.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Systemic immune microenvironment and regulatory network analysis in patients with lung adenocarcinoma, PMID: 35116596". It is recommended to quote this

article.
Reply: We have modified our text as advised (see Page 15, line 489-491).

6) There are many detection methods for cell proliferation, migration and invasion. If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.
Reply: In the current study, we employed CCK-8, wound healing, and Transwell assays to investigate cell proliferation, migration, and invasion. In the subsequent research project, we plan to expand our experimental approach by incorporating xenograft tumor models in nude mice and EdU cell proliferation assays in tumor cells to further explore the related content.

7) It is suggested that the internal mechanism of TWF1 and immune cells should be added to the discussion.
Reply: We have modified our text as advised (see Page 11, line 341-347).

Reviewer B

1) First, the abstract needs some revisions. The background the authors need to indicate the potential clinical significance of this research focus and why there is a need to focus on TWF1. The methods need to describe the clinical variables and prognosis outcomes in the database and how the prognostic role was ascertained. The results need to quantify the findings by reporting the expression levels, correlation coefficients, and accurate P values. The conclusion needs comments for the clinical imoplications of the findings.
   Reply: We downloaded the clinical information for all lung adenocarcinoma patients from TCGA, which includes survival time, survival status (deceased or alive), T, N, M, and stage classifications. By merging the expression levels of TWF1 in the samples with the corresponding clinical information, we were able to analyze and draw conclusions regarding the relationship between TWF1 expression levels and patient prognosis

2) Second, the introduction of the main text needs a detailed review on known prognostic biomarkers in LUAD and have comments on the limitations and knowledge gaps. It is also necessary to describe the potential strength of TWF1 in comparison to other known prognostic biomarkers, as well as the potential clinical significance of this research focus.
   Reply: We have modified our text as advised (see Page 11, line 362-364).

3) Third, in the methodology of the main text, please have an overview of the research procedures of this study and the questions to be answered by these procedures, and subsequently, describe them one by one. The authors need to explain the why they

developed the nomogram based on TWF1 and clinical factors, which seems not necessary because of no external validation sample and the only question to be answered is the prognostic role of TWF1. The authors need to perform multiple Cox regression to ascertain the independent prognostic role of TWF1.

Reply: We constructed a nomogram based on the Cox regression results. The rationale and significance of constructing a nomogram based on the Cox regression findings are as follows: Enhanced predictive accuracy: The nomogram incorporates multiple factors, such as TWF1 expression and T, N, and M stages, which can help improve the accuracy of prognosis prediction for lung adenocarcinoma patients compared to using individual factors alone. Individualized risk assessment: By integrating multiple variables in a single graphical representation, the nomogram allows for a more personalized assessment of patient prognosis, enabling clinicians to develop tailored treatment plans based on individual risk profiles. Ease of use and interpretation: Nomograms are user-friendly tools that can visually represent complex mathematical models. They facilitate straightforward interpretation of the results by both clinicians and patients, which can aid in the decision-making process. Comparative assessment: The nomogram can be used to compare the relative importance of different factors in predicting patient outcomes, providing insights into the potential therapeutic targets and avenues for future research. Overall, the construction of a nomogram based on Cox regression results is valuable in improving the prediction of patient prognosis, facilitating individualized treatment strategies, and guiding future research on potential therapeutic targets.

4) Please also describe the clinical factors and prognosis outcomes in the database used. In statistics, please describe the statistical software and ensure $P<0.05$ is two-sided.

Reply: We conducted our analysis using the relevant clinical information (e.g., age, gender, survival time, and staging) of lung adenocarcinoma patients obtained from the TCGA database. The complete clinical information has been uploaded along with the original data.

We have modified our text as advised (see Page 8, line 239-245).