

PEER-REVIEW REPORT

Name of journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 83617

Title: Integrated analysis of single-cell and bulk RNA-seq establishes a novel signature for prediction in gastric cancer

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03768438

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-02-02 12:01

Reviewer performed review: 2023-02-08 09:51

Review time: 5 Days and 21 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Novelty of this manuscript	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Good <input checked="" type="checkbox"/> Grade C: Fair <input type="checkbox"/> Grade D: No novelty
Creativity or innovation of this manuscript	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Good <input checked="" type="checkbox"/> Grade C: Fair <input type="checkbox"/> Grade D: No creativity or innovation

Scientific significance of the conclusion in this manuscript	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Good <input checked="" type="checkbox"/> Grade C: Fair <input type="checkbox"/> Grade D: No scientific significance
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input checked="" type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous
	Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

By integrating and analyzing three scRNA-seq datasets and ten bulk-seq datasets, the authors screened out 9 genes significantly correlated with the prognosis of gastric cancer. Before this article was published, I had the following concerns: Main 1. Please elaborate on the process, parameters, and results of the variance analysis for scRNA-seq. Setting the criteria of " $\log_{2}FC > 0.5$ & $p < 0.05$ ", there are 934 genes left, why exclude lowly expressed genes? 2. Similarly, please illustrate why exclude lowly expressed genes in differential analysis of Bulk-seq. The volcano map (Figure 3D), on the other hand, marks them clearly. 3. The manuscript lacks an introduction to the screening process for so-called important genes. 4. The flow chart seems to fail to show the data analyzing and processing flow. Minor 1. The first part in the results section duplicates the content of Mast cells, but lacks the content of Chief Cells. 2. The screening criteria of difference analysis for Bulk-seq are inconsistent in the manuscript ($\log_{2}FC > 1.5$) and volcano map ($\log_{2}FC > 1$). At the same time, the colors in the volcano map seem to be unprecise. Please double-check the volcano map (Figure 3D). 3. The supplementary materials are incorrectly marked in the third part of the results section. Table S3 corresponds to

prob_1se, while prob_min corresponds to Table S4. 4. Abbreviations need to be defined when they first appear in the text. The so-called first time here is also calculated separately in the abstract, the text (from the preface to the discussion), each illustration and each table annotation. 5. The results of the two LASSO models were intersected to obtain 11 intersecting genes. Comma segmentation is missing between some genes. Please double-check the manuscript text (including symbols, singular and plural of words, etc.) to maintain the rigor of the paper.

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Peer-review model: Single blind

Reviewer's code: 04232981

Position: Editorial Board

Academic degree: MD, MSc, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Bangladesh

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: Geng-Long Liu

Reviewer accepted review: 2023-03-07 04:52

Reviewer performed review: 2023-03-08 01:31

Review time: 20 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Novelty of this manuscript	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Good <input type="checkbox"/> Grade C: Fair <input type="checkbox"/> Grade D: No novelty
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Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input type="checkbox"/> Anonymous <input checked="" type="checkbox"/> Onymous
	Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

Major Comments: 1. Are there controversies in this field? What are the most recent and important achievements in the field? In my opinion, answers to these questions should be emphasized. Perhaps, in some cases, novelty of the recent achievements should be highlighted by indicating the year of publication in the text of the manuscript. 2. The results and discussion section is very weak and no emphasis is given on the discussion of the results like why certain effects are coming in to existence and what could be the possible reason behind them? 3. Conclusion: not properly written. 4. Results and conclusion: The section devoted to the explanation of the results suffers from the same problems revealed so far. Your storyline in the results section (and conclusion) is hard to follow. Moreover, the conclusions reached are really far from what one can infer from the empirical results. 5. The discussion should be rather organized around arguments avoiding simply describing details without providing much meaning. A real discussion should also link the findings of the study to theory and/or literature. 6. Spacing, punctuation marks, grammar, and spelling errors should be reviewed thoroughly. I found so many typos throughout the manuscript. 7. English is modest. Therefore, the

authors need to improve their writing style. In addition, the whole manuscript needs to be checked by native English speakers.

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Reviewer's code: 03757295

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: United States

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: Geng-Long Liu

Reviewer accepted review: 2023-03-07 02:15

Reviewer performed review: 2023-03-14 18:12

Review time: 7 Days and 15 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
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Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous
	Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The authors analyzed three single-cell RNA sequencing data and ten bulk RNA sequencing data from the previously published gastric cancer samples. The differential genes were subjected to linear regression model and random forest classifier to identify the genes for gastric cancer diagnosis. Please see the specific comments below: Major comments: - What is the purpose of including bulk RNA sequencing data? Have you considered building a predictive model by comparing healthy with cancer epithelium from the single-cell RNA seq data? How much difference was there between the classifier derived from the combination of scRNA and bulk and the one derived from the single-cell only? - It's known that patient heterogeneity may impact the feature identification, as shown in the bulk RNA sequencing data. Did you notice any batch effects in the single-cell RNA sequencing data? In addition, what are the regressed-out features and their biological function, which could be verified by GSEA analysis. - There is a lack of validation of the predicted model. An additional experiment is critical to verify if the prediction model is valid. The possible method includes immunohistochemistry staining, real-time PCR, or in situ hybridization. I would suggest



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adding some forms of evidence to support the prediction. Minor - What are the differences across three scRNA-seq data? I recommend a more detailed discussion on the collected data and the biological differences between the samples. For example: compiling a supplementary table that includes cancer stages, phenotypes, and prognosis.

- The clustering and cell identity assignment based on the markers didn't show the cancer populations as the ones in the published articles. How are the labeling differ from the original articles included in the analysis?
- While building the classifier, was there hold-out data (normal cells v.s. cancer cells) to validate the prediction model classified the samples correctly?
- The text in the figures (Fig 2, 3, 4, 5) is small. Please ensure that the labels are legible.

I recommend accepting this article after a major revision.

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Peer-review model: Single blind

Reviewer's code: 03522829

Position: Peer Reviewer

Academic degree: PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Egypt

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: Geng-Long Liu

Reviewer accepted review: 2023-03-07 17:44

Reviewer performed review: 2023-03-20 09:37

Review time: 12 Days and 15 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Novelty of this manuscript	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Good <input type="checkbox"/> Grade C: Fair <input type="checkbox"/> Grade D: No novelty
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Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous
	Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The aim of this study is to develop an epithelial-related GC prediction model based on single-cell RNA sequencing (scRNA-seq) and bulk RNA sequencing (bulk-seq) data. Actually, the current proposal is interesting. Therefore, I recommend that the current study be published after major revisions as follows: 1- Please, validate and confirm these findings by utilizing the cancer genomic atlas (TCGA) data. 2- Could the authors highlight the significance of TCGA data to study the complex interaction of immune cells in the tumor microenvironment of cancer as well as cancer cells? reference: SnapShot: TP53 status and macrophages infiltration in TCGA-analyzed tumors. Int Immunopharmacol. 2020 Sep;86:106758. doi: 10.1016/j.intimp.2020.106758. 3- Could the authors discuss the possible mechanisms for these findings? 4- Please add a diagrammatic figure to summarize the findings

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Academic degree: MD, MSc, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Bangladesh

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: Li Li

Reviewer accepted review: 2023-04-06 21:35

Reviewer performed review: 2023-04-06 21:36

Review time: 1 Hour

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
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Peer-reviewer	Peer-Review: <input type="checkbox"/> Anonymous <input checked="" type="checkbox"/> Onymous



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<https://www.wjgnet.com>

statements

Conflicts-of-Interest: [] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Authors addressed all of my comments. The revised manuscript can be accepted for final publication.

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Reviewer's Country/Territory: Egypt

Author's Country/Territory: China

Manuscript submission date: 2023-02-01

Reviewer chosen by: Li Li

Reviewer accepted review: 2023-04-07 07:37

Reviewer performed review: 2023-04-07 07:40

Review time: 1 Hour

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
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statements

Conflicts-of-Interest: [] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors have successfully addressed all comments

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statements

Conflicts-of-Interest: [] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors has successfully answered all the questions, I have no further comments.