World Journal of Clinical Cases

Editorial office

December 15, 2023

Dear editor,

We wish to re-submit the revised manuscript titled “Identification and validation of a new prognostic signature based on cancer-associated fibroblast-driven genes in breast cancer”. The manuscript ID is 90377.

We thank you and the reviewers for your thoughtful suggestions and insights. The manuscript has benefited from these helpful comments and we look forward to working with you and the reviewers to move this manuscript closer to publication.

The revised manuscript has been edited and the necessary changes have been made (highlighted in yellow font in the revised manuscript) in accordance with the reviewers’ suggestions. Responses to all comments have been prepared and attached.

The manuscript has been preprinted on the Research square preprint platform(https://doi.org/10.21203/rs.3.rs-2740940/v1).But, this time we have improved it, including adding validation experiments and other sufficient discussions to make it more perfect.

Thank you very much for your time and consideration. We look forward to hearing from you soon.

Sincerely,

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Responses to the reviewers' comments

Reviewer #1

Thank you very much for your kindly affirmation on our work.

Comment 1.*Conclusion in abstract does not match the conclusion given in main text file.*

Reply 1:

Thank you very much for carefully reviewing our manuscript. We are very sorry for our negligence of the detail. We have made corresponding changes in our revised manuscript, now.

The contents modified are as follows: *Conclusion: We introduced a newly-discovered CAFs-associated gene signature, which can be employed to estimate BC patient outcomes conveniently and accurately.* (line 55-56).

Thanks so much.

Comment 2. *What method has been employed in the present study to see prognosis in set of patients studied? The study is a retrospective study, there is no mention of disease free survival or recurrence and its correlation with the markers studied.*

Reply 2:

K-M survival estimate was conducted to assess the relationship between the prognostic signature and the survival of breast cancer patients. In addition, the utility of the prognostic signature was validated by the receiver operating characteristic (ROC) curve.

This section is not described because the relevant data(disease free survival or recurrence) is not available in the TCGA database. In future studies, we will continue to observe this part prospectively through our own central data .

Thank you very much for your excellent suggestion.

Reviewer #2

Thank you very much for your kindly affirmation on our work.

Comment 1.

1. The authors are welcome to discuss the findings of three recent and very relevant publications:

i) Wang Y, et al. A novel signature based on cancer-associated fibroblast genes to predict prognosis, immune feature, and therapeutic response in breast cancer. Aging (Albany NY). 2023;15(9):3480-3497. doi: 10.18632/aging.204685. Epub 2023 May 4. PMID: 37142271; PMCID: PMC10449298,

ii) Huang B, et al. Construction of a Matrix Cancer-Associated Fibroblast Signature Gene-Based Risk Prognostic Signature for Directing Immunotherapy in Patients with Breast Cancer Using Single-Cell Analysis and Machine Learning. Int J Mol Sci. 2023;24(17):13175. doi: 10.3390/ijms241713175. PMID: 37685980; PMCID: PMC10487765, and

iii) Xu A, et al. Identification of prognostic cancer-associated fibroblast markers in luminal breast cancer using weighted gene co-expression network analysis. Front Oncol. 2023 May 3;13:1191660. doi: 10.3389/fonc.2023.1191660. PMID: 37207166; PMCID: PMC10191114.

Reply 1: Thank you very much for your nice suggestion. We have added some description during the "**INTRODUCTION**" section in our revised manuscript, now.

Thanks so much.

Changes in the text:

To further describe the "*STAT3 induces breast cancer growth via ANGPTL4, MMP13 and STC1 secretion by cancer associated fibroblasts*[9]*. Cancer-associated fibroblasts facilitate premetastatic niche formation through lncRNA SNHG5-mediated angiogenesis and vascular permeability in breast cancer*[10]*.High expression of TGF-a, PKMYT1 and decreased SFRP1 and SFRP2 in the fibroblasts were associated with disease recurrence, invasive disease or decreased survival*[11]." in the "**INTRODUCTION**" section (line 80-85).

Comment 2.

2. The authors are encouraged to update the references used, since the topic is rapidly gaining interest. As a paradigm, the following publications are referred:

i) Avalle L, et al. STAT3 induces breast cancer growth via ANGPTL4, MMP13 and STC1 secretion by cancer associated fibroblasts. Oncogene. 2022;41(10):1456-1467. doi: 10.1038/s41388-021-02172-y. Epub 2022 Jan 18. PMID: 35042959,

ii) Zeng H, et al. Cancer-associated fibroblasts facilitate premetastatic niche formation through lncRNA SNHG5-mediated angiogenesis and vascular permeability in breast cancer. Theranostics. 2022;12(17):7351-7370. doi: 10.7150/thno.74753. PMID: 36438499; PMCID: PMC9691361.,

iii) Fang WB, et al. Transcriptome analysis reveals differences in cell cycle, growth and migration related genes that distinguish fibroblasts derived from pre-invasive and invasive breast cancer. Front Oncol. 2023;13:1130911. doi: 10.3389/fonc.2023.1130911. PMID: 37091166; PMCID: PMC10118028.

Reply 2: Thank you very much for your insightful comments.

We have added some content in the "**DISCUSSION**" section, which are shown on lines 372–380.

The content added is as follows. *Previous reports indicated that cancer-associated fibroblast genes expression can estimate breast cancer patient prognosis and predict the responses to immunotherapy[36-38].Wang Y, et al reported that signature based on cancer-associated fibroblast genes could divide patients into low- and high-risk groups, accompanied by different OS, clinical features, and immune infiltration characteristics[36].Huang B, et al identified various heterogeneous CAF cell populations in breast cancer patients. An CAF-associated gene signature was developed to predict the responses to immunotherapeutics[37]. The five-gene prognostic CAF signature presented by Xu A, et al was effective in estimating clinical immunotherapy response[38].*

Thanks so much.

Comment 3.

3) Many figures are hardly readable; thus it is impossible to review the figures 2, 3, 5, 6, 7, 8, 10, 11, 14, S1, and S2. Therefore, the authors are kindly requested to provide figures of enhanced quality.

Reply 3:

Thank you very much for carefully reviewing our manuscript. We are very sorry for our negligence of the figures, Therefore, we use PPT to show the pictures, which improves the readability and conciseness, and makes our views more prominent. Thanks so much.

Comment 4.

Minor issues

1) The authors are requested to explicitly define overall survival. 2) The authors are kindly requested to explicitly comment on missing values and state wether they have

imputed data. 3) The authors are kindly asked to provide a Supplemetary Table with the detailed results of the LASSO analysis depicted in Figure 5A.

Reply 4: Thank you very much for your insightful comments.

1)OS is defined as the time from diagnosis to death from any cause. It was shown on lines 108.

2)The missing value occurs because the TCGA database does not provide this part of the data. Therefore, NA is substituted in the article.

3)The detailed results of the LASSO analysis has been added in the Supplemetary Table.

The detailed results of the LASSO analysis

Gene name	coef
ADPRH	-0.03841259
APOL2	-0.046797654
CXCL16	-0.006820927
IL18	-0.083097678
LSP1	-0.008731578
MYD88	-0.160611236
NPC2	-0.047418241
NR1H3	-9.35E-06
PARP12	-0.036121288
SLC43A2	-0.007804175
STX11	-0.013966887
UBA7	-0.049411743
GLIPR1	-0.136244718
GSN	-0.013562801
MDFIC	-0.081041876
NEDD9	-0.030109754
TNN	-0.133184665
BHLHE41	-0.045265736
DNAJB5	-0.299372234
FKBP14	0.320734088
XG	0.44040419

Thank you again for your kindly affirmation on our work.

EDITORIAL OFFICE'S COMMENTS

-Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright

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Reply: Thank you for the comments. The contents of picture have been replenished, which were uploaded by using PowerPoint.

Authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Reply: Thank you for the comments. The table has been modified in the article.