Peer Review File

Reviewer A

Comment 1: This manuscript contributes new knowledge about autophagy related biomarkers as a role in gastric cancer progression via immune infiltration by using various bioinformatics analysis. The study was focused to identify the autophagy markers that promote gastric cancer progression and the authors found it out as cathepsin B (CTSB). In this manuscript, the authors described that overexpression of CTSB lower the overall survival in gastric cancer patients and the potential function of CTSB was found to be in association with immune infiltrating cells.

Reply 1: We thank the reviewer for the positive comments.

Comment 2: The manuscript is quite interesting and well written. The purpose of the study, methods, bioinformatics analysis, results, and discussion parts are clearly presented and well organized. However, I would like to request some minor points to revise. The work mentioned in the manuscript must be written in past tense and it is highly recommended to check English grammar throughout the manuscript.

Reply 2: Thank you for recognizing the work and pointing out its shortcomings. We are sorry for neglecting this issue, and we will carefully revise the English grammar problems in the manuscript.

Comment 3: Title: The title of the manuscript is adequate, but please check (immune infiltrates or Immune Infiltrates)

Reply 3: We have changed the 'immune infiltration' in the title to 'Immune Infiltration'.

Changes in the text:see Page 1, line 2.

Comment 4: Abstract: The abstract is adequate and well-constructed.

Reply 4: We thank the reviewer for the positive comments.

Comment 5: Introduction: The reason for selecting the autophagy related genes as candidate gene group should be mentioned and justified well in introduction section.

Reply 5: Thank you very much for raising the question. We mentioned the connection between autophagy and cancer in the introduction section and found multiple studies reporting the association between autophagy and gastric cancer (see page 3, lines 76-85). Autophagy-related genes are expected to become new targets for intervention in the treatment of gastric cancer.So we chose autophagy-related genes as the candidate genome.

Comment 6: Methods:

Line no 97 – 99: When you describe the different groups in each gene set, it should be consistence.

Eg: (… normal and …. cancer) or (….. cancer and ….. normal) in all three datasets.

Reply 6: We have uniformly changed to (….. cancer and ….. normal) in the three datasets.

Changes in the text:see page 3, lines 101-103.

Comment 7: Line no 104: Please check whether DEGs were selected by log FC or log2 FC (because in result section, it was described with log2 FC)

Reply 7:I'm sorry, due to our negligence, this error occurred. We have now corrected it to "|log2FC|".

Changes in the text:see page 3, lines 109.

Comment 8: Line no 144: Please check and confirm the statistical analysis method (unpair or pair t-test).

Reply 8:I really appreciate you pointing out this mistake. We have now modified it to a non-paired t-test.

Changes in the text:see page 4-5, lines 153-154.

Comment 9: Results:

Line no 146: Please edit the subtitle (Eg: Identification of ….. or something else)

Reply 9: Thank you for pointing out this issue. We have modified the subtitle to "Identification of relevant DEGs in the GEO database."

Changes in the text:see page 5, line 156.

Comment 10: Line no 168: There are altogether 5 hub genes were identified by Cytoscape. But here the authors chose only CTSB as a candidate gene. Please specify the reason for choosing CTSB. And I am also curious about the log2 FC value of CTSB and the other four hub genes.

Reply 10: We are very grateful for your question, which is just what we wanted to supplement. As for the genes *HIF1A* and *EIF2AK3* (*PERK*), we found that they are overexpressed in gastric cancer tissues. However, KM-plotter analysis indicates that their overexpression is actually beneficial for patient survival, as shown in Supplementary Figures 1A-B. This suggests that they may be protective genes, so we have excluded them. As for the gene *PRKCD*, its diagnostic model prediction ability is not as good as *CTSB* (see Supplementary Figure 1C), so we have also excluded it. Finally, although the gene *HSP90AB1* does indeed exhibit good characteristics, it has been extensively studied, as in the article "Wang H, Deng G, Ai M, Xu Z, Mou T, Yu J, Liu H, Wang S, Li G. *Hsp90ab1* stabilizes LRP5 to promote epithelial-mesenchymal transition via activating of AKT and Wnt/β-catenin signaling pathways in gastric cancer progression. Oncogene." Therefore, we have decided not to further explore this gene. In the end, we have chosen *CTSB* as the candidate gene.

In addition, the log2 FC values of these five hub genes may not be exactly the same, as we used different datasets GSE79973 and GSE54129 for differential analysis. Due to the differences in these datasets, the results may be biased. These values only reflect the magnitude of differential changes. When selecting target genes, we only used the threshold of |log2 FC|>0.05 to ensure that we do not miss any target genes. The choice of 0.05 is to expand the screening range. Thank you again for your question. If you have any further questions, please feel free to contact us. Best regards.

Comment 11: Line no 176: Please change to "Figure 4C – 4D" instead of "Figure 4C – 6D).

Reply 11: Thank you very much for pointing out this issue. We apologize for the error, which occurred due to our carelessness. We have now made the necessary corrections to "Figure 4C – 4D".

Changes in the text:see page 5, line 186.

Comment 12: Figure 4: In the figure legend, please check and confirm whether Figure 4C described the protein levels of CTSB in normal oral tissue or gastric tissue.

Reply 12:I appreciate you pointing out this error. We have now modified it to "the protein levels of CTSB in normal gastric tissue".

Changes in the text: We re-uploaded Figure 4.

Comment 13: Discussion

Line no 239 – 240: The sentence above is replicated, please delete it.

Reply 13:I'm very sorry, due to our negligence, an incorrect duplication occurred when copying the manuscript, which caused this issue. We have corrected it to "In previous studies, the significant role of the apoptotic mechanism in the development of gastric cancer was emphasized." The cited reference is correct, and we appreciate you bringing this error to our attention.

Changes in the text:see page 7, line 246-247.

Comment 14: Conclusion: The conclusion part well reflects the overall findings in the manuscript.

Reply 14: We appreciate the positive comments from the reviewer.

<mark>Reviewer B</mark>

1. **All abbreviations** in figures/tables and legends should be explained. GC and ARGs in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends/footnote.

2. Figure 2A was a little distorted. Please check if it's necessary to resubmit.

3. For the figures from HPA database, please indicate each source link in the corresponding legends.

4. Figure 4: There are not *, **, and *** in the figure, however you explain them in the legends. Please remove.

<mark>(*p < 0.05, **p < 0.01,***p < 0.001,</mark>and ****p < 0.0001)

5. The p value in Figure 4A is inconsistent with that in the manuscript. Please check and revise.

<mark>****p < 0.0001</mark>). ɔles (<mark>p < 0.001</mark>).

6. Figure 6: Please revise pvalue to p value.

pvalue	p.adjust
1e-10	4.341e-08

7. Please provide Figure 6 in higher resolutions if possible.

8. Please indicate the meaning of the following symbols in Figure 8.

9. Please provide the legends of supplementary Figure 1.

10. The citation of supplementary Figure 1 is missing in the main text. Please check and revise.

Reply:

1. Thank you for your suggestion, we have completed the modification.

2. We have re-uploaded Figure 2A, please see the attachment.

3. Thank you for your suggestion, we have completed the modification.
   Changes in the text:see page 11, lines 449-454.

4. Thank you for your suggestion, we have completed the modification.
   Changes in the text:see page 11, lines 455.

5. I'm sorry, due to our negligence, this mistake occurred, but we have made the necessary corrections.
   Changes in the text:see page 5, lines 187.

6. Thank you for your suggestion. We have now uniformly changed "pvalue" to "p.adjust", and in order to provide a more intuitive understanding of the enrichment standards, we have added the NES value.
   Changes in the text:see page 6, lines 203-205 and Page 12, line 466.

7. We have re-uploaded Figure 6, please see the attachment.

8. Thank you for your suggestion, we have completed the modification.
   Changes in the text:see page 12, lines 475.

9. We have provided a new legend for Supplementary Figure 1.
   Changes in the text:see page 12, lines 477-481.

10. We supplemented the citation to Supplementary Figure 1 in the main text.
    Changes in the text:see page 7, lines 273-279.