Peer Review File

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Reviewer A

This is a well-written paper by Zehua He and colleagues exploring the possible role of TAP1 in the tumor microenvironment and its prognostic significance in gastric carcinoma (GC). Authors collected clinical datasets from on-line available platforms and analysed them with bioinformatic tools. The results were presented on 8 figures and additional supplementary figures (9). Authors concluded that TAP1-High GC patients exhibited a TME with increased infiltration of immune cell subpopulations alongside the activated immune-related signaling pathways. interestingly, TAP1-High patients were more sensitive to immunotherapy. Such up-regulation of TAP1 correlated with increased transcriptional levels of PD-L1, indicating that TAP1 may serve as a novel biomarker to predict favorable immunotherapeutic response in GC. .

The authors extensively discussed their results in relation to recent scientific literature and clinical data reports.

Paper has 55 references which are relevant to article's subject.

This is an interesting study, and clinically valuable.

Major comments:

Comment 1. Whole Results section did not undergo carefully and thorough text editing since each sentence includes doubled phrases of similar meanings. Thus, Results section is hardly eligible for reading and comprehending presented results.

Reply 1: Thank you for the professional comments. We checked the descriptions in the Results section carefully and re-written the sentences which included double phrases of similar meanings. In the revised manuscript, we have modified our text as advised (see Page 9, lines 259-268; Page 10, lines 283-286; Page 12, lines 347-350; Page 13, lines 368-377).

Changes in the text:

According to previous findings, TAP1 plays an important role of TAP1 in tumorigenesis and

progression in certain carcinomas, we then evaluated the relationship of TAP1 expression with GC's clinicopathological characteristics within the TCGA cohort. As depicted in Figure 1B, there was no significant differences with statistical significance observed among patients displaying different clinicopathological characteristics. Specifically, TAP1 expression levels did not show a statistically significant difference across patients at different clinical / TNM stages (Figure 1C-1G), suggesting that no association between TAP1 was related to any clinicopathological features within the TCGA cohort.(Page 9, lines 259-268; Page 13, lines 368-378)

Given the absence of significant differences in TAP1 expression among patients with various clinicopathological characteristics and the positive correlation between TAP1 and immune scores, we then delved into the link of between TAP1 expression and the immunological characteristics which can reflect the immune status of TME. (Page 10, lines 283-286)

Utilizing the nearest shrunken centroids method (see "Methods"), 166 patients in the GSE84437 cohort were recognized as TAP1-H group, and 267 patients were determined as the AP1-L group (Figure 5A).(Page 12, lines 347-350)

Subsequently, based on the expression levels of canonical markers, these clusters were annotated into nine distinct cell types, namely tumor cells, B cells, plasma cells, endothelial cells, epithelial cells, fibroblasts, myeloid cells, mast cells, as well as T cells (Figure 6A-6C). Given the pivotal role of T cells in anti-tumor activity, we investigated the link of TAP1 expression with the percentage of T cells. A positive relationship of TAP1 expression with the infiltration of T cells who highly expressed cytotoxic signatures, such as GNLY and PRF1, was demonstrated ($R2 = 0.32$, $p = 0.062$, Figure 6D-E), further indicating that high TAP1 expression were positively linked with the infiltration of T cells and the heightened anti-tumor activity.(Page 13, lines 368-377)

Minor comments:

Comment 2. I wish the authors had not presented their own clinical data to enrich their analysis

Reply 2: Thank you for the professional comments.

The reviewer gives an accurate summary of our work and brings forward constructive

questions. We have addressed them below. At that time, we had added our own clinical data just to verify that our results of bioinformatics analysis, which were consistent with clinical practice in the results. We had discussed CD-8 in the discussion section of the paper. But we also thought of this question, should we have in-depth discussion?

Reviewer B

Your manuscript, "Role of TAP1 in the identification of immune-hot tumor microenvironment and its prognostic significance for immunotherapeutic efficacy in gastric carcinoma", shows a series of bioinformatic analyses to evidence the participation of TAP1 in the tumor environment, immune response, and putative support to targeted therapy. Although the potential contribution of this study to their field, I would like to comment on some concerns.

Major comments

1. Please describe the methods for determining TAP1 low and high-expressing groups. According to Figure 1, it seems to be split by a median value. However, it must be described in the Methods section.

Reply 1: Thank you for the professional comments. We apologized for not making this clear in the Methods section. In our study, the TAP1 low and high-expressing groups were distinguished by utilizing the k-means clustering (k-means clustering, k = 2, default parameters) based on the transcriptional profile of TAP1 and its co-expressed genes. In the revised manuscript, we have modified our text as advised (see Page 6, lines 176-180).

Changes in the text: Based on the consensus matrices and silhouette analysis, we determined the optimal number of TAP1-related groups. Next, k-means clustering (k-means clustering, k = 2, default parameters) was performedto divide the patients of the TCGA cohort into 2 groups on the transcriptional profile of TAP1 and its CEGs.

2. Please cite all Figures and Tables in the text according to their order of apparition. For

example, "Supp Figure 3" could not be mentioned in the text before "Supp Figure 1" and "Supp Figure 2".

Reply 2: Thank you for the professional comments. In the revised manuscript, we have modified our text as advised (see Page 11, lines 307-308).

Changes in the text: On account of the consensus clustering matrices, we determined that the optimal cluster number to be two (Supplementary Figure 3).

3. Please limit the Methods section to the sole description of methods followed to run the analysis. All descriptive results must be included in the Results section.

Reply 3: Thank you for the professional comments. We removed the description of results in the Methods section to the Results section. In the revised manuscript, we have modified our text as advised (see Methods section, Page 6, lines 176-180; Results section, Page 11, 310-312).

Changes in the text: Based on the consensus matrices and silhouette analysis, we determined the optimal number of TAP1-related groups. Next, k-means clustering (k-means clustering, k = 2, default parameters) was performedto divide the patients of the TCGA cohort into 2 groups on the transcriptional profile of TAP1 and its CEGs.(Methods section, Page 6, lines 176-180)

Then, utilizing k-means clustering, the 389 individuals within the TCGA cohort were stratified into two subgroups (Figure 3B-3C): the TAP1-H group (n=169) as well as the TAP1- L group (n=220). (Results section, Page 11, 310-312)

Minor comments

4. It seems that the manuscript will edited. However, edition marks were not removed. Several clauses need to be edited. For example, "As shownThe results," (line 241), "To be specific,Specifically," (line 244), "GivenofConsidering" (line 247), etc.

Reply 4: Thank you for the professional comments. We apologized for not carefully checking the contents. In the revised manuscript, we have modified our text as advised (see Page 9, lines 259-268).

Changes in the text: According to previous findings, TAP1 plays an important role of TAP1 in tumorigenesis and progression in certain carcinomas, we then evaluated the

relationship of TAP1 expression with GC's clinicopathological characteristics within the TCGA cohort. As depicted in Figure 1B, there was no significant differences with statistical significance observed among patients displaying different clinicopathological characteristics. Specifically, TAP1 expression levels did not show a statistically significant difference across patients at different clinical / TNM stages (Figure 1C-1G), suggesting that no association between TAP1 was related to any clinicopathological features within the TCGA cohort.

5. Please add TAP1-H or TAP1-L labels to Figures5G and 5H.

Reply 5: Thank you for the professional comments. In the revised manuscript, we added the TAP1-H or TAP1-L labels to Figure 5G and 5H.

6. Figures 6D, 6E, and 7B are showing correlation profiles, then they must be described as they are (not as an association),

Reply 6: Thank you for the professional comments. In the revised manuscript, we have modified our text as advised (see Page 13, lines 368-377; Page 13-14, lines 389-391).

Changes in the text: Subsequently, based on the expression levels of canonical markers, these clusters were annotated into nine distinct cell types, namely tumor cells, B cells, plasma cells, endothelial cells, epithelial cells, fibroblasts, myeloid cells, mast cells, as well as T cells (Figure 6A-6C). Given the pivotal role of T cells in anti-tumor activity, we investigated the link of TAP1 expression with the percentage of T cells. A positive relationship of TAP1 expression with the infiltration of T cells who highly expressed cytotoxic signatures, such as GNLY and PRF1, was demonstrated ($R2 = 0.32$, $p = 0.062$, Figure 6D-E), further indicating that high TAP1 expression were positively linked with the infiltration of T cells and the heightened anti-tumor activity. (Page 13, lines 368-377).

Besides, the increase of TAP1 expression was accompanied by the enrichment of immune cells in the PRJEB25780 IT cohort ($R2 = 0.45$, $P = 0.002$, Figure 7B). (Page 13-14, lines 389-391).