Peer Review File

Reviewer Comments

Reviewer A

Comment 1: It is not clear from the manuscript, how the glycolysis score was derived and claimed as novel. The authors need to include the details of the input data used to construct the signat˜ure.

Reply 1: Thank you very much for your suggestion, we have added some details to explain how the glycolysis score was obtained. To my knowledge, this is the first study to derive glycolysis score in all TCGA tumor types by performing single-sample gene set enrichment (ssGSEA) analysis according to the glycolysis gene set from the Molecular Signatures Database, so we claimed it as novel glycolysis score.
Changes in the text: See page 4, line 114-118

Comment 2. In prognostic value calculation for pan-cancer analysis, the authors have used different patient stratification for this analysis. The analysis should be performed with similar cut-offs before making any claims.

Reply 2: Thank you very much for your suggestion. We have added some explanations about stratification in the text. We uniformly used the best cutoff value to stratify the included patients in the survival analysis. The optimal cutoff value was automatically calculated by the "survival" and "survminer" R packages and varies due to different glycolysis scores in different tumors.
Changes in the text: See page 4, line 122-125

Comment 3. Were there any mutations in glycolytic genes and what was their correlation with the glycolysis signature?

Reply 3: Thank you for your question. We have analyzed the single nucleotide variation (SNV) and copy number variation (CNV) of the12 glycolysis key genes. The results were demonstrated in Figure 10B and Figure 11A. Meanwhile, we have described these results in more detail in the text. Since mutation analysis is a descriptive analysis of each glycolysis key gene, it is not related to glycolysis score.
Changes in the text: See page 7-8, line 285-288 and line 293-298.

Comment 4. Since the glycolysis score is associated with immune checkpoint inhibitor expression, did the glycolysis score has any prognostic value in predicting response to chemotherapy?

Reply 4: Thank you very much for your valuable suggestion. Considering that the expression of immune checkpoints mainly affects the outcome of immunotherapy, we further analyzed the relationship between glycolysis score and clinical outcomes of patients receiving immunotherapy.
Changes in the text: See page 2, line 41-42 and 50-51; page 3, line 68-69; page 5, line

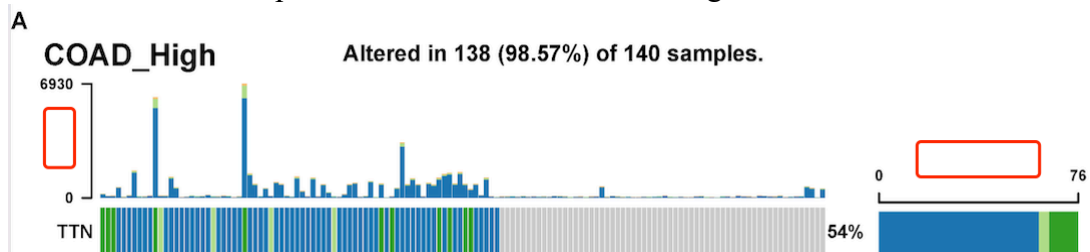1. Figures
   (1) Please provide an explanation for "*" "**" "***" "****" and "ns" in Figure 2.
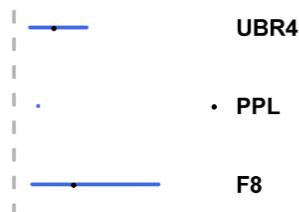   (2) Please add the description of the x-axis in Figure 4A-F.

   (3) Please provide an explanation for "*" "**" "***" "****" in Figures 5, 6.
   (4) Please add a description to indicate the meaning of red box in Figure 6.
   (5) Please add the description of the bellow two axes in Figure 8A and B.

   (6) Please provide an explanation for "**" "***" in Figure 8 legend.
   (7) Please check Figure 8C legend. There are 12 genes in Figure 8C, but why 10 in the legend?

in low glycolysis score COAD subpopulations. Genes are ranked by mutational frequency. (C) Top 10 significantly different mutated genes between two groups were displayed in a forest plot.

   (8) Please check if Figure 8C is uncompleted.

   (9) Please indicate the full name of "DEGs", "FDR", "FC", "SNV", "wt", "OS", "PFS" in Figure 10 legend.
   (10) Please provide an explanation for "*" "**" "***" in Figure 15 legend.
   (11) There is no "***" but "****" in Figure S1, please check and revise.
Reply 1: Thank you for your detailed review.
(1) We have provided an explanation for "*" "**" "***" "****" and "ns" in Figure 2 legend.
(2) We have added the description of the x-axis in Figure 4A-F.
(3) We have provided an explanation for "*" "**" "***" "****" in Figures 5, 6.
(4) We have described the meaning of red box in Figure 6.
(5) We have added the description of those two axes in Figure 8A and B.
(6) We have provided an explanation for "**" "***" in Figure 8 legend.
(7) We apologize for our carelessness. We have revised the Figure 8C legend.

(8) We have checked the Figure 8C and confirmed that it is completed. Because the value is very small, the picture presented looks like that.

(9) We have defined all abbreviations in each legend.

(10) We have provided an explanation for "*" "**" "***" in Figure 15 legend.

(11) We apologize for our carelessness. We have revised the Figure 12 legend.

Changes in the text: Figure 4 and Figure 8. Page16-18, Line633-709.

2. Please check the author mentioned in the following sentence, which are inconsistent with the corresponding reference. It is suggested to use the same name as the reference to avoid confusion. Please check through and revise.

- To explore the effects of glycolysis on the tumor microenvironment, three biological processes connected to TME reported by Zheng et al.(14, 15) were applied,

  14. Zeng D, Li M, Zhou R, Zhang J, Sun H, Shi M, et al. Tumor Microenvironment Characterization in Gastric Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures. Cancer Immunol Res. 2019;7(5):737-50.

  15. Li Z, Li Y, Shen L, Shen L, Li N. Molecular characterization, clinical relevance and immune feature of m7G regulator genes across 33 cancer types. Front Genet. 2022;13:981567.

- … and the MSI data were obtained and processed using the pipeline described by Russell et al(17).

  17. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO Precis Oncol. 2017;2017.

Reply 2: Thank you for reading our article carefully and putting forward very valuable comment. We have checked the references and modified our inappropriate statement in the text.

Changes in the text: Page4, Line147-148; Page5, Line159.

3. Please check if more references should be cited in the following sentence since you mentioned "studies".

- Previous studies have shown that the transformation from oxidative phosphorylation to glycolysis in tumors could suppress apoptosis by weakening mitochondrial function(4).

Reply 3: Thank you for your detailed review. We have cited corresponding references in the text.

Changes in the text: Page3, Line89.