

Peer Review File

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Reviewer A

The manuscript by Wang and colleagues presents a pure database analysis but no translational aspects. Moreover, it suffers from several drawbacks including

- No up-to-date classification
- No cell type specific data

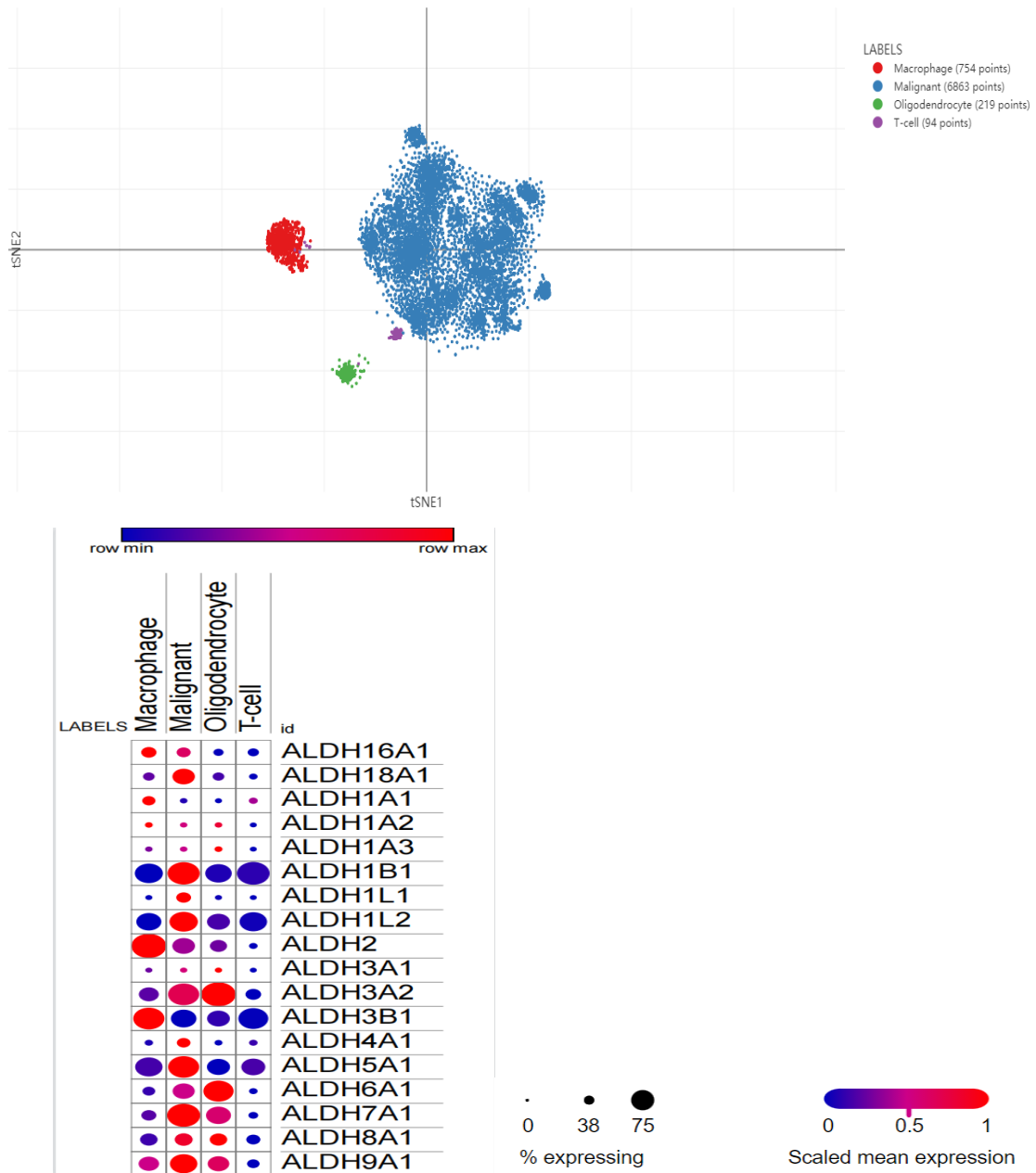
Wang and co-workers present an interesting approach to sub-classify ALDH enzymes based on expression data in glioma databases. The work suffers, however, from several drawbacks.

Comment 1: It presents a pure database analysis. The authors must show which cell-types (tumor cells, microglia, lymphocytes etc.) express different ALDH enzymes. The statement “Only samples with more than 80% of tumor cells were used.” on page 4 line 90 cannot be correct. How did the authors verify this statement? In GBM IBA-1 positive microglial cells comprise almost 50% of the cells of the tumor tissue.

Reply 1: Thanks for your valuable comments. We have performed novel analysis on different sub-classify ALDH enzyme in distinct cell types based on a public scRNA-seq dataset from 28 patients with GBM (GSE131928). These 24,131 single cells were divided into four cell types including macrophages, malignant cells, oligodendrocytes and T cells. The dot plot showed that a majority of ALDH isoforms, including ALDH16A1, ALDH18A1, ALDH1B1, ALDH1L1/2, ALDH3A2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDH8A1 and ALDH9A1 were enriched in malignant cells, while ALDH16A1, ALDH2, ALDH3B1 tended to enrich in tumor infiltrating macrophages. ALDH3A2 and ALDH6A1 were enriched in oligodendrocytes. Interestingly, all of the ALDH isoforms expression in tumor infiltrating T cells were lower than those in malignant cells.

In page 4 line 90, we made an unclear description. “ Samples with more than 80% of tumor cells” means the samples used in our analysis contained more than 80% of tumor tissue compared with normal cells.

Changes in the text: We have added a new figure in supplementary figures. And we have modified Page 4 line 90 in the manuscript to make the description more clear.



Comment 2: The authors compared different tumor types. Glioma is just a heading term like „lung cancer “and includes several different tumor types. IDH-mutant Astrocytomas/Oligodendrogliomas present an absolutely different biology when compared with IDH-wildtype glioblastomas. Moreover, the WHO 2016 classification is obsolete, since several updates by the cIMPACT-NOW system have been performed so far and the guidelines of the WHO 2021 classification are also available. IDH-mutant glioblastomas (e.g. Fig. 3, Fig. S3) or IDH-wt astrocytomas no longer exist.

Reply 2: Thanks for your careful review. We have updated the nomenclature for glioma classification according to the fifth edition of WHO classification of tumors of the central nervous system.

Changes in the text: We have revised the terminology used in the text and modified the figure 3 and figure S3.

Comment 3: No data about treatment are presented. Did all patients receive the same adjuvant therapy? What was the MGMT status of the tumors? Age, gender etc.?

Reply 3: Thanks for your comments. All of the patients were followed up every three months by phone or clinic. The clinical and follow-up information of all patients in this study are available on the CGGA website(<http://www.cgga.org.cn/>).

Changes in the text: We revised the Materials and Methods part of the text.

Comment 4: The authors must show the basis of the ALDH/immune response correlation either by tissue stains or experimental data.

Reply 4: As mentioned in the manuscript, most immune functions were negatively related to ALDH expression. Only distinct isoforms of ALDH were positively related with the immune functions, such as ALDH1A3 and ALDH16A1. Lots of previous studies have elucidated such a relationship with experimental data. Please refer to

1. Yuan Yunfeng, Wang Lin, Ge Di et al. Exosomal O-GlcNAc transferase from esophageal carcinoma stem cell promotes cancer immunosuppression through up-regulation of PD-1 in CD8 T cells.[J]. Cancer Lett, 2021, 500: 98-106.
2. Li Xiaohao, Fan Kunpeng, Liu Yafang et al. Administration of a recombinant ALDH7A1 (rA7) indicates potential regulation of the metabolite and immunology pathways in Atlantic salmon infected with *Aeromonas salmonicida*. [J]. J Fish Dis, 2021, 44: 961-977.
3. Maccalli Cristina, Parmiani Giorgio, Ferrone Soldano, Immunomodulating and Immunoresistance Properties of Cancer-Initiating Cells: Implications for the Clinical Success of Immunotherapy. [J]. Immunol Invest, 2017, 46: 221-238.

Changes in the text: We revised the discussion and references parts of the text.

Reviewer B

The manuscript uses two large datasets including the TCGA to examine the importance of Aldehyde dehydrogenase (ALDH) in brain cancer patients. The techniques of unsupervised clustering is appropriate and the author considers other characteristics in the prediction of survival. The writing needs some work to improve the clarity and the authors needs better reflect on the limitations.

Comment 5: The author should re-read the citation Yan et al., 2009, included below is a snippet from the paper that describes the frequency of different mutations. The interesting factor of IDH mutation is not the frequency but the predictive ability of the mutation to demonstrate survival in LGG. “Most (80%) of the anaplastic astrocytomas and glioblastomas with mutated IDH1 or IDH2 genes also had a mutation of TP53, but only 3% had alterations

in PTEN, EGFR, CDKN2A, or CDKN2B (Table 2). Conversely, anaplastic astrocytomas and glioblastomas with wild-type IDH1 and IDH2 genes had few TP53 mutations (18%) and more frequent alterations of PTEN, EGFR, CDKN2A, or CDKN2B (74%) (P<0.001 for both comparisons by Fisher's exact test). Loss of 1p and 19q was observed in 45 of 53 (85%) of the oligodendrocytic tumors with mutated IDH1 or IDH2 but in none of the tumors with wild-type IDH genes (P<0.001 by Fisher's exact test). In the introduction first paragraph, this sentence and the one that are not easy to understand within the paragraph. "The clinical and basic research about IDH mutation appears continuously and has inspiring progress, such as the inhibitor of IDH[5]." What is appears continuously, do you mean is commonly researched in the literature? What is the link between IDH mutation and immunotherapy? These ideas deserve more writing to flush out your logic.

Reply 5: Thanks for your comments. We have revised the introduction first paragraph to make it more rigorous.

Changes in the text: We revised the text as advised.

Comment 6: In the results section 3.1, the authors should name the different clusters outside of the figure and supplemental GO1-5 and KEGG1-5.

Reply 6: Thanks for your comments. We added names in the text.

Changes in the text: We revised the text as advised.

Comment 7: The author should consider reframing the findings in terms of the limitations. This is a big data analysis of the ALDH and is not experimental therefore it cannot provide the functionality of the isoforms. Further testing needs to be conducted to verify these findings. The authors do not address any limitations in the discussion, clearly the approach is an issue.

Reply 7: Thanks for your careful comments. The limitation of our functional analysis approach has been added in the text.

Changes in the text: We revised the text as advised.

Comment 8: The Conclusion at the end of the paper should do a better job summarizing all the important findings.

Reply 8: Thanks for your comment. The conclusion part of the text has been revised.

Changes in the text: We revised the text as advised.

Minor

Comment 9: Issue with sentence, attracted should be in the present participle (attracting) or

change are to have to keep the sentence in past tense. “Therefore, new effective therapeutic methods for gliomas are increasingly attracted researchers’ interest, including immunotherapy and small molecule inhibitors therapy [2,3].”

In the second paragraph of the introduction consider simplifying the writing, including combining sentences (“Targeted therapies to interrupt the 65 ALDH metabolic pathway are one of the directions of its research. However, the effect of ALDH inhibitors is lacking [11].”). The authors also use a lot of adverbs like however, furthermore, etc.

In the methodology, Statistical analysis section the first sentence the word all fully is capitalized. “ALL statistical computations and figure drawing were performed with R packages (ggplot, pheatmap, and survival), GraphPad Prism 7.0, and Microsoft Office 2016 pro.”

Figure 1 C it is hard to see the different classifications of the functions as some of the colors are very similar. The author should consider changing the colors and making the width of the column wider to be able to discern the different groups.

There was a typo for WHO classification and appears as whom. “Compared to WHO II grade gliomas, the expression of ALDHs in Group I and Group II was significantly lower in whom III, but all ALDHs in Group IV showed the opposite trend.”

The Author should check how they label Figures throughout the paper, in most cases they are capitalized. There is a place in the results that does not follow this, “As shown in figure 6 and figure S5”.

The authors do not need to say “and so on” when they list examples when starting a list with “such as”. “Recent studies have also confirmed that ALDH was a novel stem cell marker for various solid tumors, such as glioma, lung cancer, breast cancer, cholangiocarcinoma, and so on [9,10,14-16].”

Reply 9: Thanks for your comment. Those defective parts of the text have been revised.

Changes in the text: We revised the text as advised.