Peer Review File

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Reviewer A

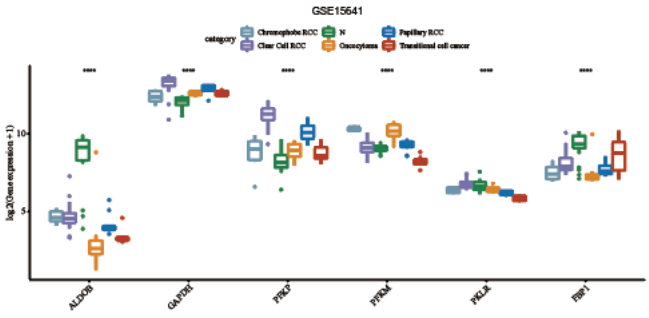
General Comments

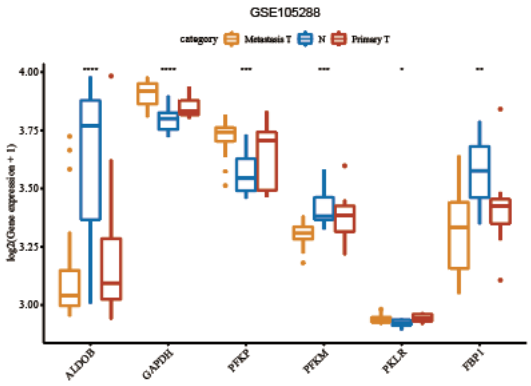
All the presented results were obtained by database search only. For the manuscript to be suitable for publication, the results should be validated. My specific comments are as follows.

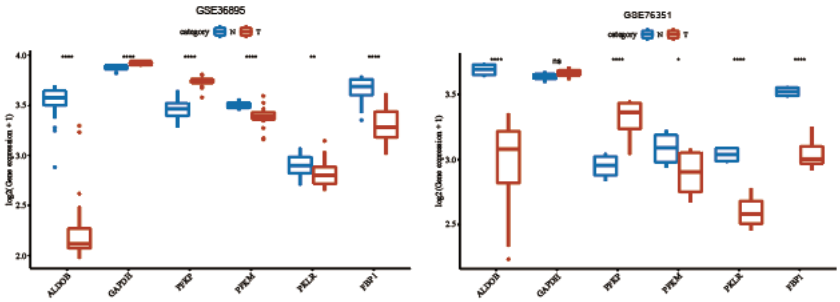
Major Comments

1. The authors should validate the results using their own cohort.

Reply: I am so sorry that there are no any ccRCC patient in our center. But we validate the results again in 4 cohorts (GSE105288, GSE36895, GSE15641, GSE76351), including 3 different types, which showed similar results in our presented results.







Changes in the text: No change in the text.

2. It is also preferable to investigate the roles of the molecules in renal cancer proliferation by in-vitro experiments.
Reply: Thanks a lot for your constructive advice. We detected the cell proliferation ability of ALDOB in Caki-1 cell lines by CCK8 experiment, which showed that the capacity of proliferation was increased in ALDOB knockdown cells.
Changes in the text: We added some data... (see Page 9, line 179-182)

Minor Comments

1. Please spell out all the abbreviations at their first use both in the abstract and in the text.
Reply: According to your suggestion, we spell out all the abbreviations at their first use both in the abstract and in the text. We also list all the Abbreviations after the abstract.
Changes in the text: We have modified our text as advised (see Page3, line 47-55)

2. Table 1: Redundant.
Reply: We deleted the Table 1.
Changes in the text: We have modified our text as advised (see Page 7, line 132)

3. LL. 52-54: Needs citation.
Reply: We added the citation to supported the claim.
Changes in the text: We have modified our text as advised (see Page 4, line 65)

4. LL. 65-73: Needs citation.
Reply: We added the citation to supported the claim.
Changes in the text: We have modified our text as advised (see Page 4, line 82-85)

5. LL. 102-106: Needs citation.
Reply: We added the citation to supported the claim.
Changes in the text: We have modified our text as advised (see Page 6, line 117)

Reviewer B

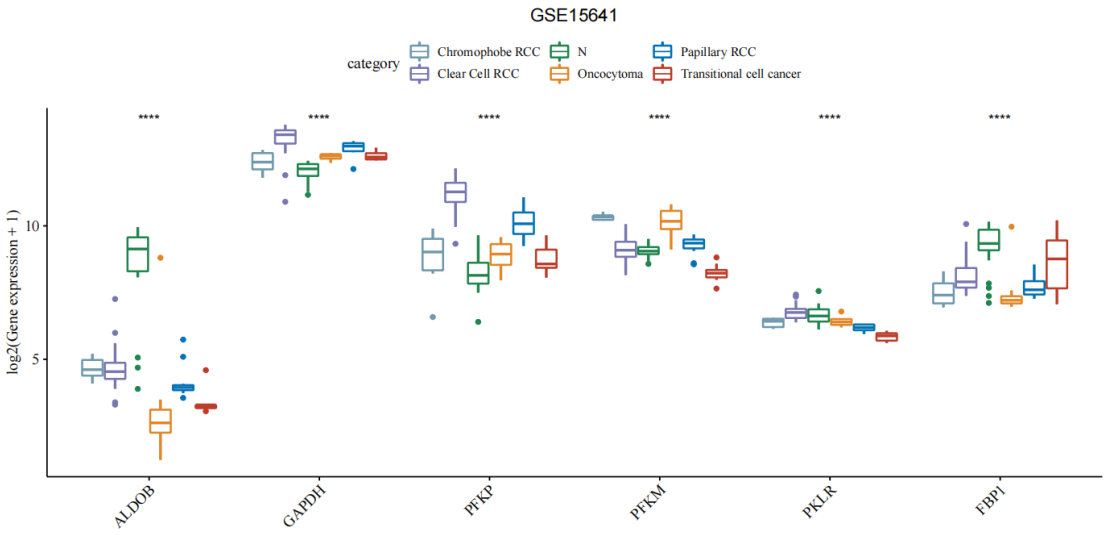
The authors used the GEO registry dataset to search for prognostic genes by bioinformatic analysis. In the search for genes differentially expressed in normal tissues and RCCs, the authors found that ALDOB significantly correlated with survival among the five genes common to the two GEO data sets. Furthermore, they report that the five genes with protein-protein interaction with ALDOB are significantly correlated with survival. This content is useful information. But there are some errors in the results, and need to be revised as follows.

Revision

1. It has been previously reported that low expression of ALDOB is associated with poor prognosis in ccRCC, but these papers are not cited. The authors should introduce the reports because it is already known. For example, the following are mentioned: 1. Jun Wang, Qi Wu & Jianxin Qiu, Accumulation of fructose 1,6-bisphosphate protects clear cell renal cell carcinoma from oxidative stress. Laboratory Investigation volume 99, pages898–908 (2019), 2. Huang H, Zhu L, Huang C, Dong Y, Fan L, Tao L, Peng Z, Xiang R, Identification of Hub Genes Associated With Clear Cell Renal Cell Carcinoma by Integrated Bioinformatics Analysis.Front Oncol. 2021 Sep 30;11:726655.
Reply: Thanks for your valuable comment. We introduced these two pieces of literature in the discussion.
Changes in the text: We have modified our text as advised (see Page 11, line 240)

2. There is no description of the association between ALDOB and the 5 genes that have been shown to have protein-protein interaction by STRING. It should be described how the increase or decrease of each gene is related to the variation in ALDOB expression.
Reply: Thanks very much for your insightful advice. We performed the qRT-PCR experiments to investigate the downstream targets of ALDOB. The results showed that the expression of GAPDH were upregulated and the level of PFKP, PFKM, PKLR, and FBP1 were downregulated in the ALDOB knockdown cell lines.
Changes in the text: We have modified our text as advised (see Page 10, line 205-208)

3. GSE40435 analyzed gene expression in frozen ccRCC tumour and adjacent non-tumour renal tissue, while GSE15641 included samples of ccRCC as well as papillary RCCs and chromophobe RCCs. Since the survival analysis targets ccRCCs, it would be better to focus on ccRCCs by analyzing GEO data that included a larger number of ccRCCs. Alternatively, if multiple types are targeted for analysis, differences of gene expression variation by RCC type should be described.
Reply: We deeply appreciate your instructive comments. We analyzed the GSE15641 cohort and found that the expression of ALDOB were significantly higher in the non-tumour renal tissues than that in the multiple types of RCC, including ccRCCs, papillary RCCs, chromophobe RCCs, transitional cell cancer, and oncocytoma.



Changes in the text: No change in the text.

4. There are several errors in the notation of GAPDH results; on page 2, line 41, GAPDH is written as AGPDH. In the Results, page 9, lines 178-181, the following sentence, seem to be incorrect. “the survival rate in the ccRCC patients who confer high expression of GAPDH, PFKP, PFKM, PKLR, and FBP1 was significantly increased compared to patients with low expression of ALDOB by log-rank test (P= 0.000346, P=0.00146, P=0.0494, P=0.0116, P=0.0000501, respectively).”
Reply: Thanks for your comment. We corrected the spell mistakes and the sentence.
Changes in the text: We have modified our text as advised (see Page 2 and 10, line 42, 200-204)