Peer Review File

Article information:

The authors performed a bioinformatic analysis of ENC1 expression and function in stomach adenocarcinoma (STAD) and colon adenocarcinoma (COAD).

The manuscript presented is simple, easy to read, well written, the results presented are novel but not shocking. In addition, there is little information on ENC1 in relation to the two cancers studied and the authors are aware of the limitations of their study, but their conclusions are sound.

Nevertheless, there are some points to consider:

1. Neither in the text nor in the figure caption is the difference between Figure 4A and 4B specified.

(Reply: we add into the legend of the Figure 4 that * indicates the diference.)

2. It should be specified with which genes the Gene function enrichment analysis was performed.

(Reply: we add into the text that the DEGs of the high/low expression group divided by ENC1 was performed by Go and KEGG.)

3. Likewise, it is not specified if the analysis of the PPI network was performed.
(Reply: we add into the text that the PPI network was constructed in the STRING database by predicting the protein that interacted with ENC1)

The study analyzed the expression of ENC1 in gastric and colon cancer tissues using data from The Cancer Genome Atlas (TCGA) database. Researchers found that ENC1 expression was higher in tumor tissues compared to normal tissues in both gastric (STAD) and colon (COAD) cancers. However, high expression of ENC1 did not significantly affect patients' prognosis and survival time. Functional enrichment analysis showed that ENC1 was mainly involved in neuroactive ligand-receptor interaction, and the PPI network indicated its role in regulating neurite formation and neural crest cell differentiation. Although there was no significant correlation between ENC1 and the prognosis of gastrointestinal cancer, the gene was found to be significantly associated with immune cell infiltration levels.

There are several potential limitations and drawbacks to the study that authors need to acknowledge.

First, TCGA data, which comes from various countries or laboratories, may not be up-to-date

or fully representative of tumor gene expression due to sequencing technology or quality issues.

Second, the study used the median value as the demarcation line between high and low expression of ENC1, which could have introduced data bias.

Third, the conclusions drawn from the data analysis need further experimental validation to confirm their accuracy and relevance.

Lastly, the study provides limited information on the potential molecular mechanisms by which ENC1 may interact with other proteins or pathways in gastric and colon cancer.

Additionally, the authors should expand or confirm their analysis by considering similar studies and utilizing other databases, such as the one referenced by DOI: 10.1097/MEG.0000000000002349.
They should also analyze protein interactions of ENC1 using the CytoHubba plug-in within Cytoscape software, and assess immune infiltration by employing the Tumor Immune Estimation Resource.

Reply: We are aware of the limitations and shortages of our studies, which we have already discussed in the text, please check page 9/line 26-34. We should have utilized multiple data sources to further development the conclusion, however, the results of this study were still solid and meaningful. Due to too few genes in the network, we didn't further use Cytoscape to identify the key hub genes. We didn't expand our immune infiltration analysis, which could be further explored in the future studies.
Changes in the text: NA

Reviewer C

1. Please check the below Keyword. You choose it as a Keyword but it cannot be found in the main text.

> 22   **Keywords:** Ectodermal-neural cortex 1; gastric cancer; colon cancer; gastrointestinal
> 23   neoplasms↵

Reply: we remove the keyword.

2. Please indicate the Online link for TCGA database.

> 19   RNA sequence (RNA-seq) clinical expression profile data were obtained from stomach
> 20   adenocarcinoma (STAD) and colon adenocarcinoma (COAD) samples in the TCGA
> 21   database. The count data, fragments per kilobase million (FPKM) data, and survival

Reply: we have already offered the link in line 20 / Page 4 for the database to download: https://xenabrowser.net/datapages/. Please check that.

3. Please add the statement "The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013)." **in both the "Methods" section of Main Text and the "Ethical Statement" section of Footnote.**

Reply: we have added that in Page 6/Line 17, Page 10/Line 22.

4. Please state whether there are any **Funding and Acknowledgements** to declare in your manuscript.

Reply: we have added that in Page 10/Line 24.

5. Figure 1:
Please check the meaning of ***. Is it correct? Should it be "P<0.001"?
There are no *, ** in Figure 1, but they were indicated in the legend. Please revise.

> 5   **Figure 1** *ENC1* expression levels in different tumor types: (A) gastric cancer; (B)
> 6   **colon cancer;** the number of dot indicates the diference significance between the
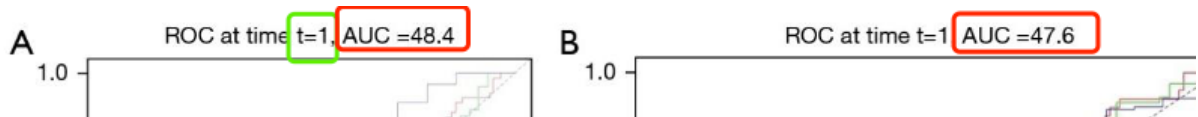> 7   two comparing group, *indicates P<0.1, **indicates P<0.01, ***indicates P<0.0001.

Reply: Revised

6. Figure 2:
What is the meaning of t=1?
And please check the data of AUCs. Are they correct?
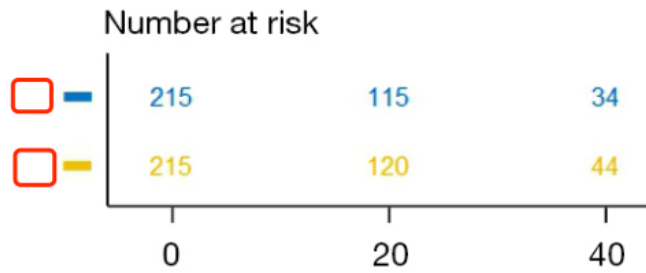Please indicate the full name of "AUC" in the legend.

A ROC at time t=1, AUC =48.4   1.0
B ROC at time t=1 AUC =47.6   1.0

Reply: t=1 stands for "year-1". Added that in the legend.   The data is the percentage num.

7. Figure 3:
1) Please remove the unit %.

% 1.00
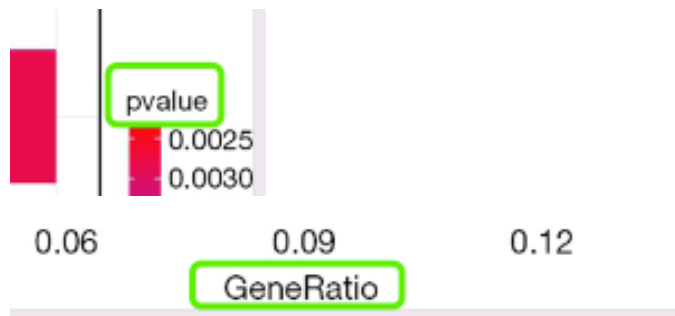ility 0.75

2) Please indicate "Low" and High" below.

Number at risk

8. Figure 4:
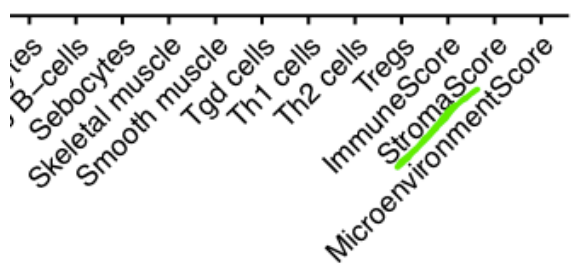1) Please revise "pvalue" to "p value" and "GeneRatio" to "Gene Ratio".

2) Please indicate the full name of "DEGs", "GAB" in the legend.

Reply : revised in the Figure 4-revised.jpg. "GABAergic synapse" is a pathway specific name which can not be separated.

9. Figure 5:
1) Please indicate the meaning of *, **, *** and ns in the legend.
2) There is a spelling mistake.

Reply: We add the meanings in the legend. The word "stromaScore" was outputed directly from the "xcell" package. We don't think it is a spell mistake.

110. Figure 6:
Figure 6 is not clear enough. Please resubmit it in higher resolution.

Reply: We updated the ENC1 search on String database, and found the network updated. So we downloaded the high resolution PNG and updated the text too.