Peer Review File

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Reviewer A:

In this manuscript, "CAP2 contributes to tumorigenesis in gastric cancer by targeting transcription factor SOX9". Actually, they demonstrate that role of CAP2 in gastric cancer. I thought that the paper well established. Therefore, I will give some suggestions.

Comments:

1. is AGC right? -> I didn't find that AGC cell line, so please check about the name of cell line. Usually, using AGS cell line.

Response: Thank you very much for your advice. Because of our spelling mistakes, we made some corrections (AGC ⟶AGS). The new picture is attached with the revised comments.

2. The authors were missing IRB number. (ex This project was approved by the Ethics Committee (No XXXXX).)

Response: Thank you very much for your advice. We have added the relevant IRB number in Footnote.

3. It is not clear why the authors chose Sox9 as the regulator of CAP2. Description on the results and discussion.

Response: Thank you very much for your advice. We predicted that there were several Sox9 binding sites near the promoter region of CAP2 through Jaspar database. Therefore, we speculated that Sox9, as a transcription factor, may participate in the regulation of CAP2 expression.

4. If possible, CAP2 overexpression experiment will likely be needed. It will be help to understand this paper.

Response: Thank you very much for your advice. In our future study, we will focus on the mechanism of CAP2 overexpression on gastric cancer

Reviewer B:

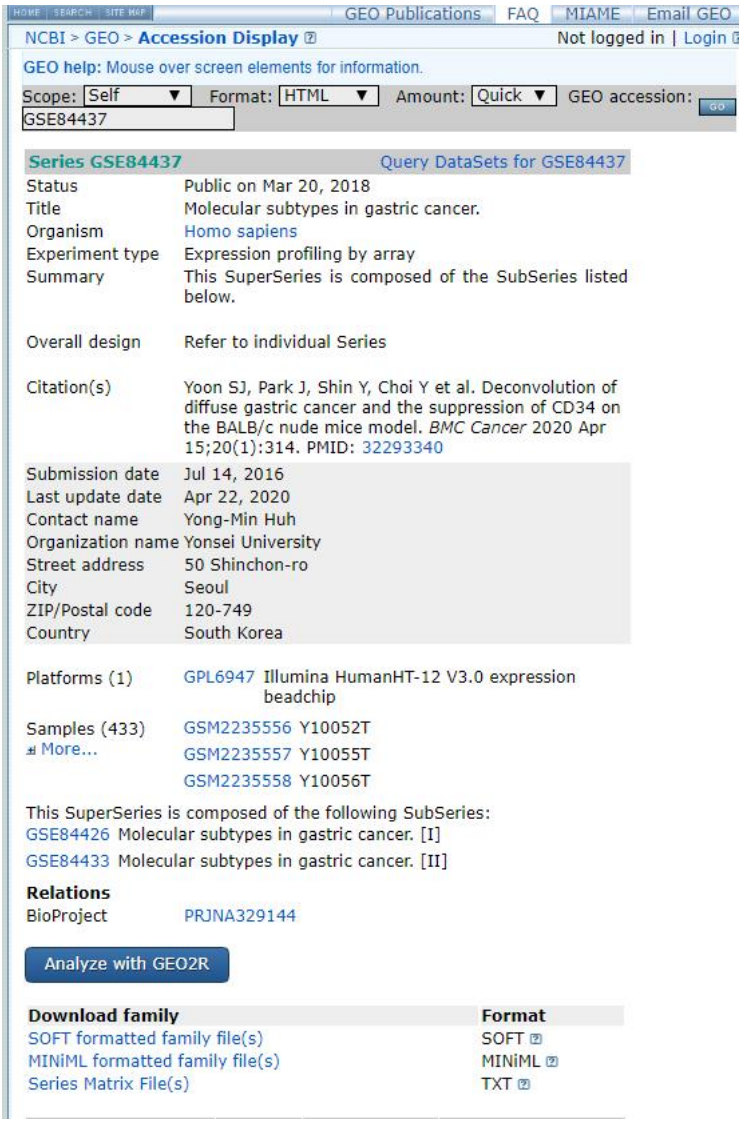
The manuscript under consideration lacks cohesiveness and has several methodological flaws.

The major weaknesses of the study are related to:
- the fact that the putative role of CAP2 as a biomarker in Gastric Cancer is based on bioinformatics analysis that is neither detailed nor characterized in the manuscript in terms of how it was performed and which criteria and tests were applied. The main data was retrieved from the GEO public repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi) and scarcely and inadequately explained regarding how it was analyzed. Another public dataset GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=CAP2) was also used in the figure 1A as a "sort of validation" without further explanation in any section of the manuscript;

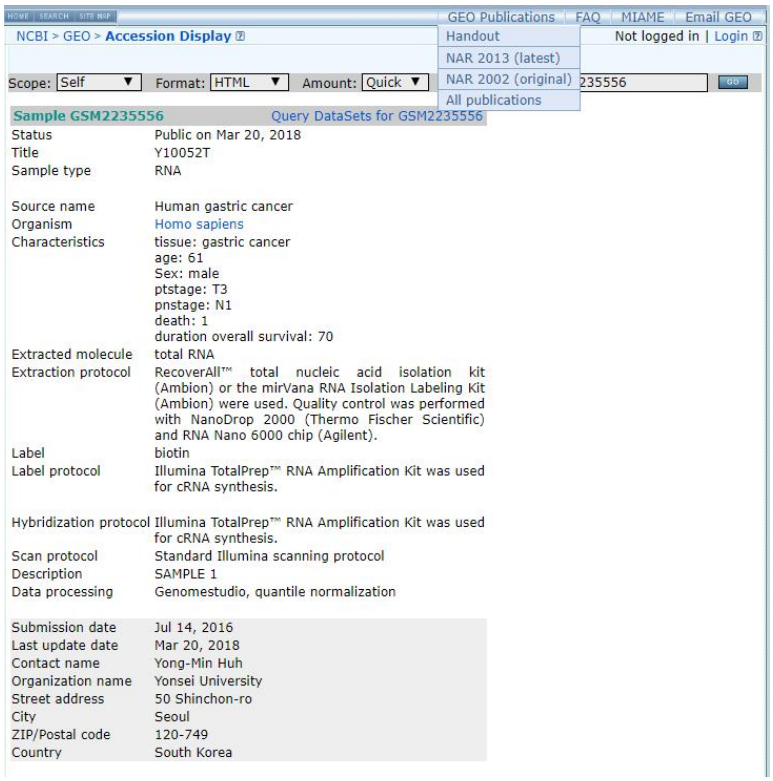
Response: Thank you very much for your advice. Firstly, the Limma package was employed to screen out the differentially expressed genes (DEGs) with $|logFC| > 1$ and false discovery rate (FDR) < 0.05 from GSE84437 datasets. Those DEGs with $logFC > 1$ and $FDR < 0.05$ were deemed as upregulated, and those genes with $logFC < -1$ and $FDR < 0.05$ were deemed as downregulated. So, we obtained 642 upregulated genes, and then we filtered out 172 genes that significantly associated with survival outcomes (log-rank $P < 0.001$). Moreover, we performed the univariate and multivariate Cox analysis to select out 82 hub genes from the 172 candidates, which can be performed to independently predict the survival time of GC patients. Combined with literature search and gene function analysis, CAP2 was selected as the gene for further study.

- besides the lack of detailed bioinformatics analysis, no detailed characterization of the patients' series is presented;

Response: Thank you very much for your advice. We can search for details of the GSE dataset through the online website (https://www.ncbi.nlm.nih.gov/geo/).



For example, for GSM2235556 Y10052T, we can click on the “Samples (433)” column to get the details of this patients’ information.



- the use of a transiently transfected gastric cancer xenograft model to evaluate the effect of genes in tumor growth, considering that siRNAs silencing doesn't last 28 days, as would be easily proven by IHC against CAP2 and SOX9;

Response: Thank you very much for your advice. Although siRNA transfection is short-term, it has already affected the cell proliferation ability in the early stage. After transplantation, it can lead to the change of cell proliferation ability, and then affect the number of cells in the initial stage, and finally detect the size of the tumor.

- is not conforming to recognized procedures to describe the methodology to be understandable and allow repetition. For the sake of example:
o No sufficient details of the transient transfection of cell lines with siRNAs is presented, which is relevant to evaluate the xenografted silenced cells (eg time-point post-tranfection at which the cell line was inoculated; no IHC for CAP2 and SOX9 in xenografts at the end of the experiment; reference to the cell line inoculated only in the legend);

Response: Thank you very much for your advice. Although siRNA transfection is short-term, it has already affected the cell proliferation ability in the early stage. After transplantation, it can lead to the change of cell proliferation ability, and then affect the number of cells in the initial stage, and finally detect the size of the tumor.

- the rationale for the experiments and the methodology used is not explained;

Response: Thank you very much for your advice. Due to the limited space of this paper, we give a brief overview of methodology

- inflate statements concerning the findings and their experimental support.

Response: Thank you very much for your advice. We have carefully worded our conclusive statements in the manuscript in order to not overly inflate the implications of our findings.