

## Peer Review File

**Article Information:** <http://dx.doi.org/10.21037/jgo-20-234>

### 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/>).

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Scope:  Format:  Amount:  GEO accession:

**Series GSE84437** [Query DataSets for GSE84437](#)

Status Public on Mar 20, 2018  
 Title Molecular subtypes in gastric cancer.  
 Organism [Homo sapiens](#)  
 Experiment type Expression profiling by array  
 Summary This SuperSeries is composed of the SubSeries listed below.

Overall design Refer to individual Series

Citation(s) Yoon SJ, Park J, Shin Y, Choi Y et al. Deconvolution of diffuse gastric cancer and the suppression of CD34 on the BALB/c nude mice model. *BMC Cancer* 2020 Apr 15;20(1):314. PMID: [32293340](#)

Submission date Jul 14, 2016  
 Last update date Apr 22, 2020  
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Platforms (1) [GPL6947](#) Illumina HumanHT-12 V3.0 expression beadchip

Samples (433) [GSM2235556](#) Y10052T  
 # More... [GSM2235557](#) Y10055T  
[GSM2235558](#) Y10056T

This SuperSeries is composed of the following SubSeries:  
[GSE84426](#) Molecular subtypes in gastric cancer. [I]  
[GSE84433](#) Molecular subtypes in gastric cancer. [II]

**Relations**  
 BioProject [PRJNA329144](#)

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

NCBI > GEO > Accession Display	
Scope: Self	Format: HTML
Amount: Quick	235556
<b>Sample GSM2235556</b> <a href="#">Query DataSets for GSM2235556</a>	
Status	Public on Mar 20, 2018
Title	Y10052T
Sample type	RNA
Source name	Human gastric cancer
Organism	<i>Homo sapiens</i>
Characteristics	tissue: gastric cancer age: 61 Sex: male ptstage: T3 pnstage: N1 death: 1 duration overall survival: 70
Extracted molecule	total RNA
Extraction protocol	RecoverAll™ total nucleic acid isolation kit (Ambion) or the mirVana RNA Isolation Labeling Kit (Ambion) were used. Quality control was performed with NanoDrop 2000 (Thermo Fischer Scientific) and RNA Nano 6000 chip (Agilent).
Label	biotin
Label protocol	Illumina TotalPrep™ RNA Amplification Kit was used for cRNA synthesis.
Hybridization protocol	Illumina TotalPrep™ RNA Amplification Kit was used for cRNA synthesis.
Scan protocol	Standard Illumina scanning protocol
Description	SAMPLE 1
Data processing	Genomestudio, quantile normalization
Submission date	Jul 14, 2016
Last update date	Mar 20, 2018
Contact name	Yong-Min Huh
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Country	South Korea

- 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-transfection 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.