RE: 41441, entitled **Overexpression of G protein-coupled receptor 31 as a poor prognosticator in human colorectal cancer**

Dear editor,

Thank you for reviewing the above-referenced manuscript submitted earlier to your office. We would like to take this chance to express our appreciation to you and colleagues.

In accord with your and the Reviewer's comments, the manuscript has been revised accordingly, and the changes have been highlighted in Yellow in the revised manuscript. We feel that the revised manuscript has been strengthened by your and the Reviewer's suggestions and comments, and we are very appreciated of your and their time and effort. A point-by-point response to the reviewers' comments and suggestions has been prepared and follows this cover letter.

If there are any questions or problems for our revised manuscript, please feel free to contact us.

Sincerely yours,

Zou Yifeng

On behalf of all co-authors

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Responses to comments

Reviewer #1:

> What is the difference between the training group and the validation cohort from the point of view of the methodology? Why are the results of training presented at all? How reliable are they? What time does the training take?

Response:

Cases in the training cohort were first collected from the First Affiliated Hospital of Sun Yat-sen University from January 1996 to December 2008 with up to 10 years follow-up. In order to test and verify the reliability of the results of the training cohort, we then analyzed cases in different centers using the same method, and got almost the same conclusion from the validation cohort.

So, there was no difference between two cohorts in methodology. And the results of training cohort are reliable after verification in different center.

Thank you!

Reviewer #2:

In the original article of Yu-Ming Rong et al. the authors examined the expression of GPR31 in tissue microarray made of CRC tissue samples by immunohistochemistry. They found significant differences in the number of patients in pM classification between low and high expression groups. They found five-year survival and tumor-free survival rates of patients in the low expression group to be higher then in the high expression group. They identified GPR31 expression as an independent predictive factor of CRC patient survival. The study is well designed and well presented. However, some points need revision: - GPR31 and related signaling pathways are included in tumor proliferation/apoptosis and invasion. What can be the explanation that GPR31 expression showed no significant correlation with any of the histological characteristics (such as differentiation, grading) of the tumor samples? - Are there any Western blot data of GPR31 expression in the observed CRC samples? Does it correlate to the IHC data? - Was any detectable GRP31 expression in the blood samples of CRC patients? If yes, what was the source of it? - The authors wrote

in the discussion: "Results of the present study showed that GPR31 expression in colorectal cancer tissue was significantly higher than in normal mucosa and that GPR31 expression levels are closely related to distant metastasis of tumors, which are consistent with findings reported in previous studies." Please, indicate the references. After major revision I suggest to accept the manuscript for publication.

Response:

1. The progression of the tumor is related to various characteristics of the cancer cells, including differentiation, proliferation, apoptosis, migration, invasion, drug resistance, stem cell-like phenotype, etc. We are not quite sure which characteristic is most regulated by GPR31. A small sample size may also be one of the reasons for these, and the difference may be found by expanding the sample size.

2. Paraffin specimens were used in this study and we did not perform WB.

3. Only paraffin specimens of patients were used in this study. We didn't detect GRP31 expression in the blood samples.

4.This conclusion is quoted from: *The G protein-coupled receptor GPR31 promotes membrane association of KRAS. J Cell Biol. 2017 Aug 7;216(8):2329-2338. PMID: 28619714*. It will be the ref. [54] in our latest revised version.