Dear Editor,

Thank you for carefully reviewing our manuscript previously titled “High PNPLA8 expression as a biomarker for poor prognosis of colorectal cancer” for possible publication in the World Journal of Gastrointestinal Oncology. We are grateful to you and your reviewers for their constructive critique. We have revised the manuscript, highlighting our revisions in red. and have attached point-by-point responses detailing how we have revised the manuscript in response to the reviewers' comments below.

Reviewer #03479334:

This is a retrospective study with PNPLA8 expression as a biomarker for poor prognosis of colorectal cancer. I have the following comments.

1) The authors described that the median follow-up time of the patient cohort was 46.1 months. What was the actual range of follow-up periods? Please give the shortest and longest follow-up periods.

Response:

The range of follow-up periods is from 5.1 month to 107 month （IQR=32.9-59.5）. 91.8% patients were followed for more than 24 months. We have updated this data in our manuscript.

2) The study is listed as running from May 2008 to November 2012, which is more than 10 years ago. It's hard to say because we don't know the longest follow-up period, but even if that's the case, the cases studied are old.

Response:

The longest follow-up period is 107 months. The median follow-up time of the patient cohort was 46.1 months（IQR=32.9-59.5）.

Although these cases were not the latest, the surgical and chemotherapeutic treatment for those colorectal cancer patients enrolled in this study had not

changed much from today. And these cases can reflect the treatment of colorectal cancer to a certain degree. Paraffin blocks from these colorectal cancer cases were carefully stored in a cool, dry specimen storage room at 4°C. Before starting the immunohistochemistry experiment, these wax block tissues were cut and made into tissue chips. The staining results of these tissue chips are not significantly different from those of recent paraffin sections. As a retrospective study, our research has its own limitations, and more prospective studies are needed in the future to verify the reliability of our results.

Reviewer #05263678:

This interesting study identifies PNPLA8 immunoreactivity as a possible prognostic factor for colorectal cancer.

1. The manuscript would benefit from review and editing by a native English speaker.

Response:

This document certifies that the manuscript entitled "High PNPLA8 expression as a biomarker for poor prognosis of colorectal cancer" was proofread an edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the qualified scientific editors at MedSci (CODE:0529-7A6F-3694-E4C2-AC37), all of whom are native English speakers. We will carefully recheck this manuscript and thanks a lot for reminding us.

2. In the paragraph on "correlations between PNPLA8 expression and clinicopathological parameters", the third sentence beginning "These samples were categorized…" suggests that only the 689 specimens with some PNPLA8 expression were categorized into low and high expressers and compared. However, table 2 suggests that all were compared (presumably categorizing the low expression and zero expression together. This should be categorized.

Response:

Sorry for the ambiguity. In this study, low-expression PNPLA8 includes samples with negative PNPLA8 expression. We have revised this paragraph in the article to avoid misunderstanding.

3. What does it mean to say that 751 were successfully stained? Were there other tissue blocks that were not successfully stained and thus discarded from the study? If so, how many?

Response:

We strictly evaluated the staining results of all samples. Among them, the tumor tissues of 66 samples were not successfully stained. The main reasons for the unsuccessful staining were: 1. The samples on the tissue chip fell off. 2. Excessive dyeing. 3. Tumor tissue chips only fixed normal tissues, etc. These samples that did not meet the criteria were removed from the study. The remaining 751 samples are still representative.

4. The last sentence of paragraph 1 of the discussion is an overstatement. These results do suggest that PNPLA8 may be prognostic, but there are no data presented here to suggest a functional role in carcinogenesis or disease progression.

Response:

Thanks a lot for pointing out this description problem in the article. We have revised this sentence.

5. Why do the authors think that this is not relevant for right sided colon cancer?

Response:

In the subgroup of the right colon, we analyzed the survival of the high and low expression groups of PNPLA8 and found no difference (P=0.7057). Based on this, at least in our set of data, the expression of PNPLA8 has no significant effect on the survival of patients in the right sided colon cancer.

6. How was informed consent obtained from patients in this retrospective study? Please clarify.

Response:

Our hospital had been committed to establishing a tissue sample library since 2005. Each patient had signed an informed consent form before undergoing surgery and expressed willingness to donate samples for scientific research. This study was completed based on tissue chips made from these retained tissue samples.

7. What was the minimal, median, and longest follow up? How were these patients followed? How many were lost to follow up and how was their data handled?

Response:

The actual range of follow-up periods is from 5.2 month to 107 month (median follow-up time =46.1 months； IQR=36.9-60.9). We use outpatient follow-up combined with telephone follow-up to track the patient's condition. Approximately 42 patients (5.6%) were lost to follow-up within 2 years, and their last follow-up time was recorded and included in the study as censored data.

8. The authors indicate that this has previously been implicated as a marker for colon cancer in other studies. What is the novelty here?

Response:

Research on the molecular of PNPLA8 in colon cancer is still in short of further investigation, and for now the mechanism of PNPLA8 on colorectal cancer is still controversial. The innovation of this study is that we found a new independent prognostic factor for colorectal cancer and CRC with a high PNPLA8 expression conferred survival impairment.

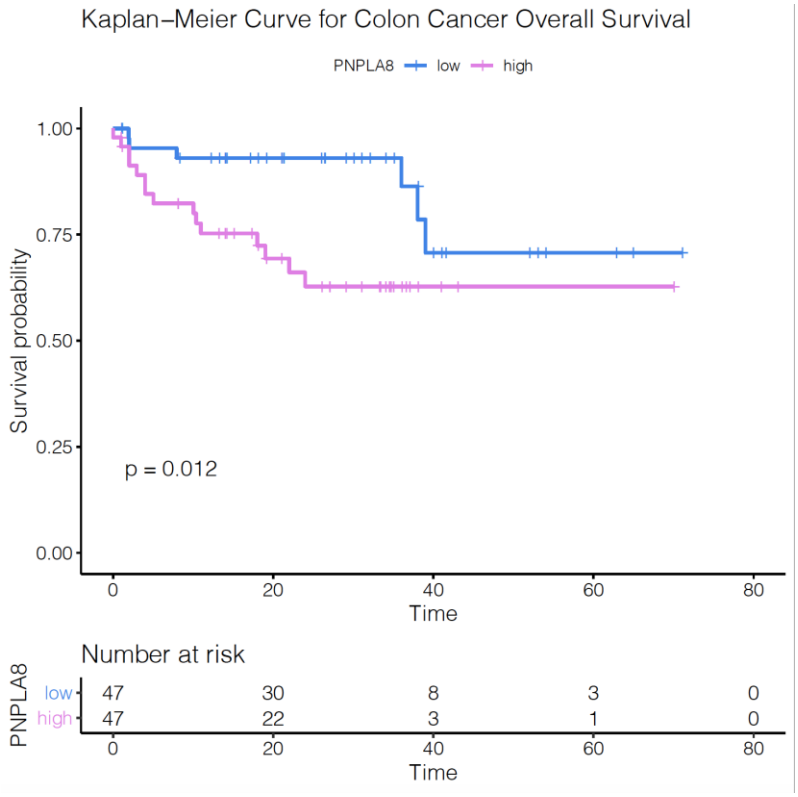
Reviewer 05330707:

1. There were two major problems with this study: first, the number of patients studied was small, and second, PNPAL8 expression was evaluated only by immunohistochemistry. The authors should investigate PNPAL8 expression in colorectal cancer using TCGA and other databases.

Response:

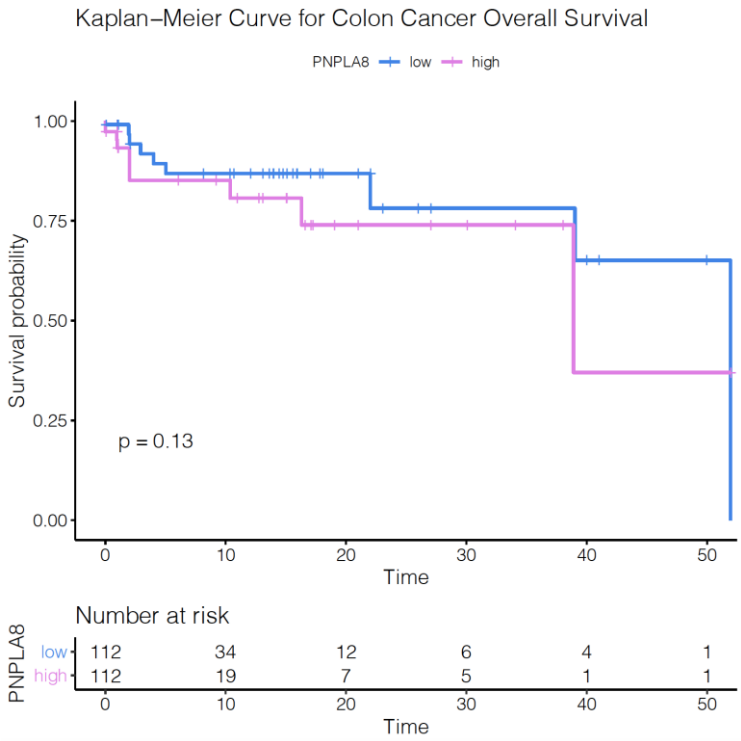
First, with such a sample size, the power value is >0.90 and the α value is <0.05, which has sufficient statistical power to support this conclusion. Of course, we will consider supplementing prospective data from multi-centers in the future to further validate this molecule

Second, we do have investigated PNPLA8 expression in other database like Clinical Proteomic Tumor Analysis Consortium (CPTAC). This is a database based on the application of large-scale proteome and genome analysis, or proteogenomics. And this is the result we found:



This result from CPTAC database support our findings on protein expression level.

We also investigate the database of TCGA, we find a similar trend (not significantly different):



Further investigation is still needed.

2. The authors should evaluate PLA2G6 (GVI; PLA2; INAD1; NBIA2; iPLA2; NBIA2A; NBIA2B; PARK14; PNPLA9; CaI-PLA2; IPLA2-VIA; iPLA2beta), not PNPAL8(MMLA; IPLA2G; IPLA2-2; iPLA2gamma; PNPLA-gamma). This is because many of the references cited (ref. #21, #22 and #24) relate to PLA2G6.

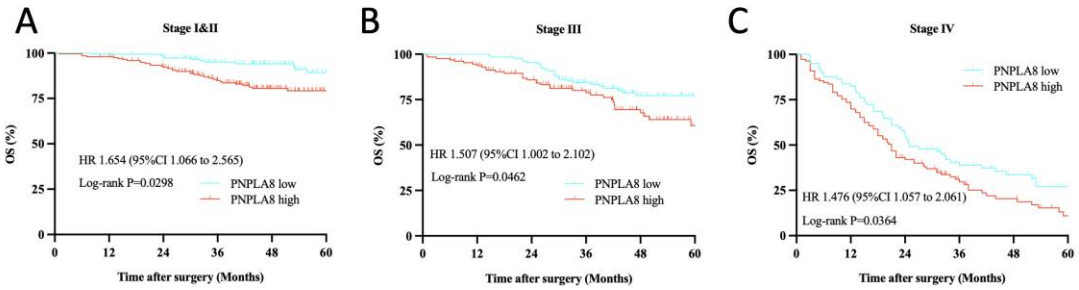
Response:

Thank you very much for pointing out the problem with the references cited in this article. Due to my carelessness, I cited inappropriate literature. I made changes to the manuscript and applied the correct citations.

3. Patients with lymph node metastases are classified as stage III, while those with distant metastases, such as lung or liver metastases, are classified as stage IV. In addition, treatment and prognosis are very different between stage III and stage IV. Therefore, stage III and stage IV colorectal cancer patients should be distinguished as a separate group to study the impact of PNPLA8 expression.

Response:

According to your reliable suggestion, we separated stage III and stage IV patients into two groups. And we observed similar trends in both two groups.



4. Please describe the adjuvant and palliative chemotherapy received by the patients enrolled in this study.

Response:

Among 353 stage I-II patients, 92 patients received adjuvant chemotherapy in

accordance with the guidelines. 255 patients did not receive chemotherapy, and 6 patients had missing data.

Among 207 stage III patients, 166 patients received adjuvant chemotherapy, 11 patients did not receive postoperative adjuvant chemotherapy, and 30 patients had missing data.

Among the 191 stage IV patients, 164 patients received palliative treatment, and 27 patients had missing data.

Thank you for your consideration and further review of our manuscript. Please do not hesitate to contact us with any further questions or recommendations.

Yours Sincerely,

Jianmin Xu