Thank you for your letter and for the reviewer's comments concerning our manuscript entitled "Analysis of unfavorable prognostic biomarker of APC in period-T4 gastric cancer". These comments are all valuable and very helpful for revising and improve our paper, as well as the important guiding significance to our research. We have carefully reviewed the comments and have revised the manuscript accordingly. Our responses are given in a point-by-point manner below:

Reviewer 1:

The authors described APC is the poor prognostic factor of T4 gastric cancer. This study is a well written, and interesting to readers of the relationship between APC-high and various factors as genes , miRNA and DNA methylation. However, the results are not well discussed and some parts should be improved with more explanations and discussions.

1. The definition of APC-high and low APC-low should be clearly described.

Answer: Yes, The definition of APC-high and low APC-low should be described in detail, we have corrected it. (page 7-8, line 197-211)

2. I can't understand why APC-high expression is associated with poor prognosis only in T4 tumors, not in T1-T3. Are there any big differences in T3 and T4 in terms of tumorigenesis? I speculate that T4 tumors are more associated with peritoneal dissemination. How about markers metastasis ability?

Answer: This is an interesting question. The findings of the study are also beyond my expectations. Firstly, all the GC patients were subjected to OS and RFS analysis, there was no significant relationship between APC^{high} and APC^{low} in OS and RFS analysis (Figure 1A, B). Because APC is a known gene which serves as an antagonist of the tumorigenesis Wnt signaling pathway to

cancer cell proliferation, metastasis, invasion, adhesion, and activation. The no difference between APC^{high} and APC^{low} in OS and RFS analysis caused our interest to continue research. Secondly, we analyzed the association between APC expression and prognosis in GC different stage, period-TNM, and grade, respectively. We found that there was significant relationship between APC^{high} and APC^{low} in OS and RFS of period-T4 (Figure 1C, D). Following, a series of research including APC-related genes, miRNA, methylation profile, the relationship between APC and GC, and APC clinically prognostic were used to verify the prognostic value of APC in period-T4. The reason why APC-high expression is associated with poor prognosis only in T4 tumors is still unknown, but this is an interesting question that deserves further study. The purpose of our study has been achieved, that is, to find APC as a diagnostic target for T4 tumors GC, and to verify my results by a series of methods.

Combining with the discussion and citation 57, I suspect that the APC is a prognostic factor in the T4 gastric cancer, but it is still a tumor suppressor. We need to figure out that APC is not the only crucial molecule involved in the Wnt, and other molecules are involved in this pathway to promote tumorigenesis. APC is involved in the mechanism of tumor inhibition of Wnt pathway, but the mechanism of action is not as strong as other molecular factors in promoting cancer, and the overall effect is still to promote tumorigenesis. We hypothesize that the APC is highly expressed in the T4 GC to fight the cancer-promoting effects of other factors, so the APC has a high expression in T4 compared to other periods.

For the speculation of reviewer, this is T4 tumors are more associated with peritoneal dissemination. We have added two important clinical characteristics, including lymph node metastasis and distant metastasis for analysis (Table 1). The result showed that lymph node metastasis was associated with the APC^{high}, which meant the GC patients of lymph node $metastasis_{2+3}$ in APC^{high} were significantly more than those in the APC^{low}.

However, there is no significant association between APC^{high} and distant metastasis. We found that tumor in AP^{Chigh} was significantly higher than those in the APC^{low}. GC patients in T4 have a greater chance of metastasis to other organs through lymph nodes, accompanied by an increase in the expression of APC. So, the speculation of "T4 tumors are more associated with peritoneal dissemination" may be right. This is one of the reasons for the elevation of APC in T4 GC, which is worthy of further study. (page 10, line 288-292; page 38-39)

3. Are there any relationship between APC-high and other factors such as TNM staging or lymph node metastasis.

Answer: Yes, we have added several clinical factors, including lymph node metastasis of N and distant metastasis of M, which are associated with APC for further analysis (Table 1). The result showed that the lymph node metastasis of gastric cancer is also closely related to the up regulation of APC. Because T 4 patients are more likely to have tumor metastasis, and tumor metastasis is related to high expression of APC. This also explains the poor prognosis with high expression of APC in T4. However, we could not found significant association between APC^{high} and distant metastasis. (page 10, line 288-292; page 38-39)

Reviewer 2:

The paper describes associations between APC expression and prognosis in period-T4 gastric cancer. High APC expression was correlated with poor prognosis, related to altered miRNA expression and consequent changes in gene expression as well as changes in DNA methylation. APC can be used as a novel biomarker for prognosis and the pathways uncovered can be targeted for treatment. Problems:

Language/writing needs considerable editing The phrase "As far as I know" (with small "i") was used, that is inappropriate for a scientific paper and in any case, there are multiple authors

Answer: Thank you for your advice. In fact, the phrase "As far as I know" is not an appropriate expression in scientific manuscript. We have corrected it. (page 5, line 136-137)

The authors can expand a bit more on the mechanisms linking APC levels with the observed effects.

Answer: The aim of our research was to determine the APC is the unfavorable prognostic biomarker in period-T4 gastric cancer. So, we conducted a series of bioinformatics method to verify our aim. However, the mechanisms between APC levels and period-T4 gastric cancer are not the important point in our study. In addition, the mechanisms between APC levels and period-T4 gastric cancer are unclear. Since tumorigenesis was the result of the interaction of genetics and epigenetic, in which genetics was mainly regulated by gene expression, while epigenetic was mainly regulated by non-coding RNA and DNA modification. So, we highlighted the important role of APC with distinctive genome-wide gene/microRNA/methylation expression and related cellular functional pathways in the pathogenesis of GC. The main purpose of associational analysis of APC and period-T4 gastric cancer is to further prove that APC can be used as a diagnostic target for gastric cancer,

not to explain the mechanismss of APC in gastric cancer. The observed effects of mechanisms linking APC levels, including APC associated with genome-wide gene analysis, APC related genes pathways, ceRNA mechanism, and relationship between APC^{high} and genome-wide DNA methylation were based on existing research results.

We have quoted a lot of other researchers' conclusion to prove our point of view. We have expand mechanisms linking APC levels with the observed effects to the part of discussion, including the interaction between proteins produced by some coding genes and APC was involved in the promotion or inhibition of tumor proliferation by Wnt pathway, and relationship between APC-dependent metabolic regulation and GC (page 17-18, line 496-517).

ceRNA was cited as linking APC to miRNA, but do the authors have any speculation on how APC affects ceRNA? As regards the levels of methyltransferases, could that be an indirect effect of miRNA changes?

Answer: This is an interesting question. We hypothesize that there was ceRNA mechanism between APC gene and miRNA based on the mRNA-miRNA regulatory network (Figure 4C). And most of miRNAs were negatively correlated with APC in associational analysis and mRNA-miRNA regulatory network. So we reasonably speculate that there is a ceRNA mechanism. CeRNA mechanism, which means that miRNA can regulate the expression of downstream genes. The high expression of miRNA can sponge mRNA to inhibit the expression of mRNA, while the low expression of miRNA relieved the adsorption of mRNA to promote the expression of mRNA. In any case, there is negative regulation between miRNA and mRNA. However, it is just that speculation needs to be further experimental verification. As for the levels of methyltransferases in the process of ceRNA by regulating miRNA, there are few reports. However, this is a good researching point, since they are all genetic level regulation.

The authors cite a reference that APC is not necessarily linked to Wnt activity in gastric cancer, but in could be linked. Consider the "just right hypothesis" for colorectal cancer. It is possible that Wnt activity is deregulated in some gastric cancers independent of APC mutation, but that higher levels of APC lower the Wnt activity to a "just right" level conducive to tumor growth? This is speculation, the point is that just because higher APC is linked to poor prognosis does not mean that Wnt signaling is not involved at all. A point to consider for the Discussion.

Answer: Thank you for reviewer's scientific comment, and we admire reviewer's scientific rigour. We have carefully read my paper and citation, and found that our discussion on this point is not strict. We described that APC is not necessarily linked to Wnt activity in gastric cancer, which is not a perfect idea. Thus, we have to figure out that APC is not involved in the Wnt doesn't mean that the gene is not necessary for this pathway. Just as reviewer's "just right hypothesis" said that: It is possible that Wnt activity is deregulated in some gastric cancers independent of APC mutation; higher APC is linked to poor prognosis does not mean that Wnt signaling is not involved at all. This is good hypothesis. In addition, we need to figure out that APC is not the only crucial molecule involved in the Wnt, and other molecules are involved in this pathway to promote tumorigenesis. For the citation, the author found that Wnt receptor (Fzd7) could promote tumorigenesis with or without mutations to APC. We misread the article as APC without the need to participate in the Wnt pathway at all. In fact, APC is involved in the mechanism of tumor inhibition of Wnt pathway, but the mechanism of action is not as strong as that of Fzd7 in promoting cancer, and the overall effect is still to promote tumorigenesis. This is reasonable answer to explain why APC regards as antitumor gene is an unfavorable prognostic biomarker for period-T4 GC. We have corrected the part in discussion. (page 16-17, line 474-482)

We try our best to improve the manuscript and make some changes in the manuscript according to the reviewer's comments. We hope that this correction will meet with approval. We look forward to hearing from you regarding our submission. If there are any shortcomings and errors, please point out and criticize, and we would be glad to respond to any further questions and comments that you may have.

Thank you and best regards

Yours sincerely