Dear Editors:

Thank you for giving us the precious opportunity for a revision of our manuscript. We appreciate the comments and suggestions from the reviewer very much. Those comments are all valuable and very helpful for revising and improving our paper. We have studied the reviewer's comments very carefully and tried our best to improve the manuscript. The followings are our point-by-point responses to the original reviewers' remarks underneath each comment.

Dear reviewer 1:

Comments to the Authors:

1. Identification of the circRNA-miRNA-mRNA regulatory network and its prognostic effect in colorectal cancer. The paper of Yin TF et al describes the role of circRNA-miRNA-mRNA regulatory network and its potential prognosis in colorectal cancer CRC. They have used databases taken from Group on Earth Observations (GEO) and the Cancer Genome Atlas (TCGA) and they have identified a competitive regulatory model among circRNAs, miRNAs and mRNAs. Moreover, they have found promising indicators that drive several processes involved in initiation and the progression of CRC cancer and affect the prognosis of CRC. For that reason, they downloaded three datasets (GSE126095, GSE41655 and GSE41657) of large-scale CRC samples in the GEO database, checked differentially expressed (DE) circRNAs, DEmiRNAs and DEmRNAs in CRC tissues compared to normal controls, and predicted the downstream target molecules of circRNAs and miRNAs. Moreover, several functional analyses were conducted to identify the underlying mechanism involved in the pathogenesis of CRC. To verify the prognostic effect of the mRNAs found above, they performed survival analysis using the gene profiles and clinical information in the TCGA database. Finally, survival-related genes were determined, and a prognostic subnetwork was developed. The novelty and significance of this manuscript are that it deals with a large scale of several circRNAs, miRNAs and mRNAs, their differential expression and their competitive interplay using sophisticated methodology. Interestingly, the authors predict that this network affects

several biological processes such as the retinol metabolic process, leukocyte chemotaxis, extracellular matrix remodeling, endoplasmic reticulum stress, ADH activity, gastric acid secretion, nitrogen metabolism and NOD-like receptor signaling pathway which might represent essential signaling pathways involved in the pathogenesis of CRC. In addition, the authors have identified three mRNA (CA2, ITLN1, and LRRC19) which are downstream effectors of this network and are significantly correlated with the clinical outcome of CRC patients. In fact, upregulation of these mRNAs might be related to better clinical outcome in CRC patients. However, their hypothesis about the role of the circRNAs, miRNAs and mRNAs network in CRC is partially supported by a series of observations based strictly on theoretical and computational approaches and statistics. Therefore, one crucial week point of this study is that it is based only on predictions and it lacks any functional biological assays to clarify the mechanism that drives these biological processes executed by circRNAs, miRNAs and mRNAs network. It is very important in the future, the authors to perform several biological assays to confirm their present findings at the molecular cellular level. Practically, the authors can conduct experiments using several colorectal cell lines and try to clarify the molecular mechanisms that drives the competitive regulation of the circRNA-miRNA-mRNA network which affects these biological processes. These studies will provide useful tools in the understanding of the pathogenesis of CRC at the molecular level having an impact on its prognosis. At last, they have to address the percentage of enrichment of differential expression of circRNAs, miRNAs and mRNAs identified and how they are cross regulated among the normal tissue, colorectal adenoma and carcinoma in the results?

Response:

Thank you very much for the good suggestions. We totally agree with you that our research has some shortcomings. First, molecular-level research in clinical samples and cell lines of CRC should be applied to validate biomarkers and clarify the actual significance of the ceRNA network and prognostic subnetwork identified in this observational study. In addition, the lack of research on the downstream target

molecules of the ceRNA network makes it difficult to completely elucidate the specific biological processes that ceRNA cross regulated in the occurrence and development of colorectal adenoma and carcinoma. We deeply appreciate your constructive comments for our further study and admire your profound knowledge. In the immediate future, we plan to conduct experiments using clinical samples, several cell lines and animal models to confirm our present findings about competitive regulation of the circRNA-miRNA-mRNA network in pathogenesis and prognosis of CRC just as you suggested.

2. It will also be necessary for the authors to add some more sentences in the discussion about the specific targets of the network during those biological processes described and involved in the pathogenesis of CRC. Which steps of these processes are affected by this network?

Response:

Many thanks for your careful review and valuable suggestions. We further discussed the specific targets and steps of biological processes affected by identified mRNAs in ceRNA network using pathway diagrams downloaded from KEGG website (https://www.kegg.jp/). CA2 participates in the process of combining water and carbon dioxide to generate carbonic acid, acting as one of the key enzymes in the pathway of proximal tubule bicarbonate reclamation and collecting duct acid secretion, and involving in gastric acid secretion and arginine biosynthesis in nitrogen metabolism. CXCXL3 is one of the downstream chemokines of NOD-NF-κB pathway in NOD-like receptor signaling pathway. ADH, as one of the key enzymes for the mutual transformation of all-trans-retinal and all-trans-retinol (vitamin A), participates in the final metabolic process of dopamine to 3-methoxy-4-hydroxy-phenylethylene-glycol in tyrosine metabolism. More details about this issue have been added in the DISCUSSION of revised manuscript.

We would like to express our great appreciation to you again for valuable comments on our paper. All of the revised portions are marked in yellow in the manuscript.

Thank you and best regards!

Yours sincerely,

Shu-Kun Yao