Cover Letter

Dear Editors and Reviewers,

Thank you very much for your efficient work and thank you for your letter and comments. Accordingly, we have revised the manuscript entitled ***"Identification and Prediction of Novel Non-Coding and Coding RNA-Associated Competing Endogenous RNA Networks in Colorectal Cancer" (Manuscript NO:42268)***, and would like to resubmit it for your consideration. We have addressed the instructive comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers' comments are listed for your consideration. We would like to express our sincere thanks to the editors and reviewers for the constructive and meaningful comments.

We are so grateful that you have offered us this opportunity to resubmit our manuscript. We hope that the revised version of the manuscript is now acceptable for publication in your journal.

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We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

Dong-Qiu Dai

Response to Reviewers

Reviewer # 1 (Number ID: 03001816)

Comment 1: This is a fine and interesting paper, and I have no problems with the bioinformatics or the general conclusions. One minor issue with the RT-qPCR. Is there any data that demonstrate whether the RNA used is DNA free? Trizol isolation is not necessarily going to get rid of all the DNA, and I do not see any indication that any subsequent DNase step was performed. Therefore, is there any negative control, such as doing the RT-PCR but skipping the RT step? If the RNA is really DNA free, then performing PCR directly on the RNA should not yield a band. Can the authors comment on this, at least? If they have any of these RNA samples left over, showing that no product is formed without the RT step would be helpful. Or do you use primers that span exon/intron junctions and thus can distinguish DNA from fully processed RNA?

Response 1 : We are grateful for your meaningful comments, and we fully understand and agree with you. In RT-qPCR, we used the PrimeScript TM RT reagent Kit (Takara, Otsu, Shiga, Japan) to convert RNA to cDNA. The Kit contained gDNA Eraser that could remove genomic DNA. We added gDNA Eraser into total RNA to get rid of genomic DNA, then performed reverse transcription with treated RNA. We have added relevant content to the article (See in the materials and methods Section)

In the design of primers, we tried to make the primers satisfy the condition of spanning exon/intron junctions. In the present research, the primers of LINC00488, IGF2-AS and GAPDH satisfied the condition of spanning exon/intron junctions. However, we did not design the primers of POU6F2-AS2 with good parameters that could meet the condition. Therefore, the primers of POU6F2-AS2 were located in the same exon. According to your opinion, we performed RT-PCR that skipping the RT step. The result showed that there was no product with the primers of POU6F2-AS2. Hopefully, this may address your concerns.

Reviewer # 2 (Number ID: 00181023)

Comment 1: The manuscript entitled “Identification and Prediction of Novel Non-Coding and Coding RNA-Associated Competing Endogenous RNA Networks in Colorectal Cancer” reports an analysis of public TCGA data using a bioinformatics approach as research tool. The only own data that the authors provide are data from three cell lines in which they show that the respective markers are “upregulated” without any functional evidence, that is functional assays. These data are incomplete and of no help and should be omitted. The alternative would be an own experimental approach including true functional studies – only these could support the database results.

Response 1：We are grateful for your meaningful comments. Our present study aims to identify and construct the competing endogenous RNAs networks in colorectal cancer with the methods of bioinformatics analysis. We performed RT-qPCR only to verify that the high-throughput results were consist with the expression in cell lines. Currently, the role of IGF2-AS, POU6F2-AS2 and LINC00488 in colorectal cancer is unclear and we would perform further research in the future. Hopefully, this may address your concerns.