Author's response to reviews

Title: miR-320b suppresses cell proliferation by targeting c-Myc in human colorectal cancer cells

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Author's response to reviews: see over
Dear Editor Mr. Ryan Relox,

We are submitting our revised manuscript entitled “miR-320b suppresses cell proliferation by targeting c-Myc in human colorectal cancer cells” (MS: 2079497320154697).

We have studied reviewer’s comments carefully and have made revision which marked in yellow in the paper. According to the comments, we have performed additional experiments and included the new data to make the revisions accordingly. We have provided the point-by-point responses below, and we think we have addressed all the concerns raised by the reviewers in our revised manuscript.

We would like to express our great appreciation to you for comments on our paper. Looking forward to hearing from you.

Thank you very much for your consideration.

Yours sincerely,

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Point-to-point responses

Responses to Reviewer# 1

Probably the authors did not understand the request regarding question 1, please substitute the sentence for the clinical-pathological data available highlighted in yellow at page 5 (lines 7-9) with “information about age, gender and tumor size were available for all patients and are listed in Table 1” that exactly states what they present in this paper..

Response: Many thanks for the positive comments. According to the comments and suggestions, we have substituted the sentence “Clinical and pathological data were available for those patients. Patients’ characteristics of clinical-pathologic features such as age, gender and tumor size were listed in Table 1” with “Information about age, gender and tumor size were available for all patients and are listed in Table 1” in our revised manuscript. We added the related description in the manuscript (Page 5, Line 7-8).
Responses to Reviewer# 2

I recognize that the authors have made an effort to make the article more relevant according the reviewers’ comments and suggestions. However, I have residual concerns on some aspects already raised in the previous revision process. The manuscript has not always been revised according my suggestions..

Response: Many thanks for the positive comments. According to the comments and suggestions, we performed additional experiments and revised our manuscript with new data. We have provided the point-to-point responses below, and hope we can address your concerns in our revised manuscript.

Question 1: The issue relative to the quality of written English for the whole manuscript remains a weak point of the present study. See for example how the original sentence on Page 3, line 10-12 has been corrected: First version: “Increasing evidence shows that post-transcriptional regulation of gene expression mediated by miRNAs acting as either tumor suppressors or oncogenes in a variety of cancers including CRC.” Second version: “Studied have shown that post-transcriptional regulation of gene expression mediated by miRNAs act as either tumor suppressors or oncogenes in a variety of cancers including CRC.” I suspect that neither the first nor the second one of the sentences expressed the sense required by the Authors (“Increasing evidence showed post-transcriptional regulation of gene expression mediated by miRNAs, acting as either tumor suppressors or oncogenes, in a variety of cancers including CRC”).

Response: Thanks for the suggestion. We have corrected the sentence “Studied have shown that post-transcriptional regulation of gene expression mediated by miRNAs act as either tumor suppressors or oncogenes in a variety of cancers including CRC” to “Increasing evidences showed that post-transcriptional regulation of gene expression mediated by miRNAs act as either tumor suppressors or oncogenes in a variety of cancers including CRC” in the revised manuscript. We added the related description in the manuscript (Page 3, Line 10-12).
**Question 2:** With respect to Figure 5A: the purpose of this reviewer was to show all the investigated conditions in a single WB image, because the samples deriving from the following conditions (empty vector pGL3 alone transfected cells; empty vector pGL3 plus negative control oligonucleotide transfected cells; vector pGL3-c-Myc alone transfected cells), represent basic “controls” of the assay, that are required to verify if c-Myc expression was modulated by negative control oligonucleotides and/or empty vectors. Please include these basic controls to the original version of Figure 5A, considering also cells transfected with mir-320b mimics and cells transfected with mir-320b mimics + pGL3-c-Myc.

**Response:** Thanks for the comments. Since overexpression of miR-320b suppressed the proliferation of CRC cells, and given that c-Myc is a direct target of miR-320b. So, we hypothesized that the inhibitory effect of miR-320b on CRC cell viability might be achieved via targeting c-Myc. In order to investigate this hypothesis, we re-designed our experiments. HCT-116 and SW-480 cells were transfected with miR-320b and/or pGL3-c-Myc, the expressions of c-Myc and Cyclin D1 were analyzed by western blotting and cell proliferation was measured by MTT assay. Our results showed that ectopic c-Myc in HCT-116 and SW-480 cells significantly increased protein expression of c-Myc and Cyclin D1 and cell proliferation, while, restoration the miR-320b expression in HCT-116 and SW-480 cells markedly inhibited expression of c-Myc and Cyclin D1 and suppressed c-Myc-induced cell proliferation in HCT-116 and SW-480 cells. Taken together, these results suggested that miR-320b could regulate CRC cells growth through targeting c-Myc. We added the related description in the manuscript (Page 12, Line 7-17, Page 22, Line 1-6).