Author's response to reviews

Title: MicroRNA-217 functions as a prognosis predictor and inhibits colorectal cancer cell proliferation and invasion via an AEG-1 dependent mechanism

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Version: 3 Date: 6 March 2015

Author's response to reviews: see over
Dear editor and reviewer:

Thank you for giving me such an opportunity to revise my manuscript. I do believe that your suggestion will make my manuscript better. Following your suggestive comments, we thoroughly revised the manuscript and we hope that the revised manuscript will meet your requirements.

Following is the response to the comments:

Reviewer (Referee 1): Jie Hong

Reviewer's report:

Major Compulsory Revisions

The authors carried out a study to illustrate the relationship between deregulation of miR-217 expression and colorectal carcinogenesis and metastasis. They also identified AEG-1 as a direct target of miR-217 in colorectal cancer. The story may have some hints for colorectal cancer diagnosis, survival prediction and treatment. However, the mechanisms of miR-217 and AEG-1 in colorectal cancer progression are still not clear.

1. The authors have shown that miR-217 significant inhibited cell proliferation both in vitro and in vivo. They just revealed that miR-217 may inhibit cell invasion in vitro, how about in vivo situation? The metastasis animal model in this paper should be performed. In addition, the author didn’t illustrate why transfection of miR-217 mimics increased cell apoptosis and blocked the cell cycle progression from Figure 3. Is there any molecule or pathway participating in miR-217-induced tumor cell apoptosis or cell cycle arrest? The author should discuss that at least.

Response:

Thank you for your kind suggestion, I do think the metastasis animal model would be better for verifying the invasion and metastasis of tumor objectively. Because of limited time and grant, it is difficult for us to supplement the experiments about metastasis animal model. If possible, we will use metastasis animal model in further research. Following your kind comments, we performed extra experiments to detect some apoptosis and cell cycle progression related proteins such as MMP2, MMP9, cyclin D1, Bcl2 and Bax, which could illustrate why overexpression of miR-217
induced cell apoptosis, cell cycle arrest and blocked invasion (The new data was shown in Figure 4C).

2. By bioinformatical analysis, the authors identified the potential miR-217 target gene as AEG-1. They showed that miR-217 binds 3′-UTR of AEG-1 and down-regulated AEG-1 expression. What is the role of AEG-1 in CRC initiation and progression? They should explore that. Moreover, it is impossible to conclude that miR-217 deregulation contributes to cancer via AEG-1. AEG-1 might be just one of the important genes regulated by miR-217. To confirm their claim outlined in the title, the authors should show that deregulation of miR-217 can no longer cause colorectal cancer in AEG-1 knockout CRC cells or mice.

Response:

Thank you for your suggestive comment. Following your suggestion, we added AEG-1 siRNA experiment as control. The results showed that similar to overexpression of miR-217, knockdown of AEG-1 significantly inhibited cell proliferation and invasion, blocked cell cycle progression and induced apoptosis (The new data was shown in Supplementary Figure S2). With regard to prove miR-217 deregulation contributed to cancer via AEG-1, we added a rescue experiment which demonstrated that overexpression of AEG-1 could reverse miR-217-induced apoptosis, cell cycle arrest, invasive and proliferative inhibition of SW620 cells (The new data was shown in Supplementary Figure S3).

3. The text has many language issues, which need correction by an English editor.

Response:

Thank you for your kind suggestion. We have made this manuscript edited by professional English editing company to make it clearly understandable.

Reviewer (Referee 2): Mogens Karsbøl Boisen

Reviewer's report:

This manuscript describes the prognostic and mechanistic role of miR-217 expression in
colorectal cancer (CRC). The authors show that miR-217 is downregulated in CRC tissue and that low miR-217 could be a negative prognostic factor. They also demonstrate an inhibitory effect of experimental upregulation of miR-217 on cancer cell line proliferation, growth and invasiveness in vitro and in vivo. Finally, they demonstrate the ability of miR-217 to target AEG-1 and show a negative correlation between miR-217 and AEG-1 in clinical samples.

The studies are well-designed and provide interesting new data to the field of microRNAs in CRC. There are many language mistakes in the manuscript and a comprehensive language revision is needed. The manuscript should be acceptable for publication if the revisions below are performed.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Provide references (web site URLs) for target prediction algorithms used (Targetscan etc.)

Response:

Thanks for your careful comment. In the Material and Methods paragraph, the sentence was described as: *miR-217-binding region was identified by TargetScan 6.2 on AEG-1*. Of course, following your suggestion, we added the web site URL of TargetScan.

2. Remove “strongly” from line 302 and line 327, “intensively” in line 313, and “great” in line 328. This is overstating the strength of the data.

Response:

Thanks for your kind suggestion. We had removed these overstated adverbs.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

3. Language editing; there are a lot of instances of misuse of words/phrases, spelling mistakes and confusing sentences. A major (professional) language correction is needed.

Response:

Following your kind suggestion, we had made our manuscript revised by a professional English
4. The authors should provide a data set with the qRT-PCR expression of miR-217, U6, AEG1 and GAPDH, survival data and clinico-pathologic data in order for reviewers and readers to validate their findings.

Response:
Thank you for your suggestion. We had provided the qRT-PCR data set and the clinic-pathologic data, as well as the survival data as supplementary information.

5. How was the pathology (cancer/normal) of the frozen samples confirmed? Please also add information about this subject in the method section.

Response:
Thanks for your kind comment. We had added the pathology confirmation of the frozen samples in the method section. (The revised result was shown in Page 6)

6. In Table 1, 19/26 patients with low miR-217 had distant metastases (=stage IV disease), but only 14/26 were listed as stage III+IV. Please explain and correct this discrepancy.

Response:
Thanks for your careful comment. I sincerely apologized for making a mistake that I recorded the figure of distant metastasis backwards. What is correct is that 7/26 patients with low miR-217 had distant metastasis, while 19/26 ones had no distant metastasis. Furthermore, we had found another two counting mistakes which had been corrected in Table 1. (The revised result was shown in Page 25)

7. The authors should also analyze the prognostic value of AEG-1 in both univariate and multivariate analysis and show if miR-217 expression has prognostic value beyond the correlation with AEG-1 expression.

Response:
Thanks for your precise comment. In the revised manuscript, we analyzed the prognostic value of AEG-1. The survival analysis showed that there was no significant difference between
AEG-1-high-expression group and AEG-1 low-expression group (The new data was shown in Supplementary Figure S1). However, for multivariate analysis, we showed that miR-217 expression still had prognostic value beyond the correlation with AEG-1 expression (The revised result was shown in Page 26)

Reviewer (Referee 3): Yaou Zhang

Reviewer's report:

Comments

It has been reported that miR-217 expression was much lower in different tumors and it plays an important role as a potential tumor suppressor. In this study, Bo Wang et al found that AEG-1, a key oncogenic factor, is a target gene of miR-217. This is interesting and novel. However, some important controls should be added to improve the manuscript.

Major Compulsory Revisions:

1. AEG-1's siRNA should be used as control.

Response:

Thank you for your kind suggestion. In the revised manuscript, we had added AEG-1 siRNA experiment as control. The results showed that similar to overexpression of miR-217, knockdown of AEG-1 significantly inhibited cell proliferation and invasion, blocked cell cycle progression and induced apoptosis. (The new data was shown in Supplementary Figure S2)

Minor Essential Revisions

2. Because of the multi target characteristic of microRNAs, AEG-1 should not be the only target, how about other targets? At least, they are should be mentioned in discussion.

Response:

Thanks for your careful comment. Following your kind comment, I had discussed this point and cited the reference in the revised manuscript as: Through bioinformation assay, we found that miR-217 targeted multiple cancer-related genes that have been reported to have a close link with
cancers, such as KRAS with pancreatic cancer[8], E2F3 with hepatocellular carcinoma[9], and DACH1 with breast cancer[38]. Interestingly, in this study, AEG-1 was predicted to be one of the target genes of miR-217.

3. In this study, near all the experiments were completed using miR-217 mimics, exception for the experiment in vivo. miR-217 stable expression cell lines should be used in some experiments in vitro to improve the quality of the manuscript.

Response:
Thanks for your suggestion. I do agree that miR-217 stable expression cell lines using in experiments in vitro will largely improve the convincing of the manuscript. However, in this study, miR-217 mimics could significantly upregulate miR-217 expression hundreds of times, which was not able to be comparable by miR-217 lentiviral vectors. We thought that using miR-217 mimics for experiments in vitro was enough to clarify the standpoint.

4. In the Figure 2B, the statistics is highly significant even though the difference of OD values at day 2 are so small, how many samples did you used in these experiments?

Response:
Thank you for your careful comment. The cell proliferation assay by CCK8 method was done with six replicates every time. This experiment was repeated three times. The effects of miR-217-induced proliferative inhibition of both SW480 and SW620 cells were remarkable actually.

In the end, we once again sincerely thank the editors and reviewers for giving such suggestive comments. We hope the revision can meet these requirements and make the manuscript more concise and understandable.

Best regards
Sincerely yours,
Shan Wang