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

Authors:

Bo Wang (bowang1986@126.com)
Zhanlong Shen (shenlong1977@163.com)
Kewei Jiang (jiangkewei@pkuph.edu.cn)
Gang Zhao (gangzhao1975@126.com)
Chunyou Wang (chyouwang52@126.com)
Yichao Yan (yanych0306@126.com)
Yang Yang (docyang_2013@163.com)
Jizhun Zhang (zhangjizhun2007@163.com)
Chao Shen (sc201@163.com)
Zhidong Gao (gaodong1234567@163.com)
Yingjiang Ye (yeyingjiang@pkuph.edu.cn)
Shan Wang (shanwang60@sina.com)

Version: 4 Date: 28 April 2015

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

Thank you for giving us such a chance to revise our manuscript. I do believe that your suggestion will make our manuscript more meaningful.

Following is the response to the comments:

Reviewer (Referee 1): Jie Hong

Reviewer's report:
I have no other questions, this manuscript can be accepted for BMC cancer publication in my opinion.

Reviewer (Referee 2): Mogens Karsbøl Boisen

Reviewer's report:
The authors have provided answers to most of the questions and have made definite improvements to the manuscript. Yet, several problems persist and some revisions have not been made satisfactorily.

Major Compulsory Revisions
1. The authors state that a professional language correction effort has been undertaken in order to correct misspelling and grammatical errors. Yet, the manuscript is still full of grammatical errors and words are missing in several sentences. These language errors significantly hamper readability and need to be corrected. The authors should consider writing a complaint to the company that performed the language editing since their work is in no way acceptable. These are some of the lines with language problems: 81-83, 85-86, 88, 90-92, 112, 114-115, 152-153, 187, 199, 228, 238, 240, 252, 263, 288, 311-312, 316, 322, 324, 332-335, 343-344, 347-348, 359-360, 364-366, 370, 374-375, 381-382.

Response:
Thank you for your kind suggestion. We have made this manuscript edited by professional English editing company again to make it clearly understandable. We hope this revised version would meet the language requirement of the journal.

2. I have re-drawn the plots in Figure 6B and 6C using the data from Supplementary file 6 (6.xlsx) and get very different results from the ones that are shown in the manuscript. I have attached these plots. When using these data, there is no significant correlation between miR-217 and AEG-1 in CRN or CRC samples. It follows that the plots cannot be based upon the data provided. Please investigate this issue and make corrections where needed.

Response:
Thanks for your careful comment. I sincerely apologize for making you confused that the data we provided were not one to one correspondence, which means in one row the expression of miR-217 and AEG-1 didn’t come from the same one sample. We have adjusted the order and make the data one-one correspondence. Furthermore, we re-drew the plots according to the new order and
found that the results from CRN samples are different from the previous one, and we have corrected it. (*The revised result was shown in page 15 and also in Figure 6B*)

**Minor Essential Revisions**

3. The description of the 2−ΔΔCt results are probably wrong. This method is defined as such: ΔΔCt = ΔCt, sample (cancer) − ΔCt, reference (normal) and ΔCt, sample is the Ct value for any sample normalized to the endogenous housekeeping gene (U6) and ΔCt, reference is the Ct value for the calibrator (normal) also normalized to the endogenous housekeeping gene.

The authors report 2−ΔΔCt results for both CRC and CRN samples. But since this requires normalization to a "normal" sample, this cannot be correct, i.e. CRN=normal samples cannot be normalized to normal. The authors have probably calculated 2−ΔΔCt values, meaning 2 to the power of minus (CRC/CRC Ct – U6 Ct). This is a valid measure, but the authors should of course change their text to reflect this measure of expression and describe how the calculations were done in the method section.

**Response:**

Thanks for your specific comment. I do agree that your description of definition of ΔΔCt is correct as one of the methods. However, our descriptions are also valid. ΔΔCt = (Ct target – Ct U6) − (Ct target’ – Ct U6’), of which target’ stand for a random detected target gene[1]. As Zhao et al. [2] described, ΔCt was calculated by subtracting the Ct of U6 or glyceraldehyde 3-phosphate dehydrogenase mRNA from the Ct of the mRNA of interest. ΔΔCt was then calculated by subtracting the ΔCt of the negative control from the ΔCt of the sample. The fold change in mRNA or miRNA was calculated according to the equation 2−ΔΔCt.


Reviewer (Referee 3): Yaou Zhang

Reviewer's report:

**Discretionary Revisions:**

Transfecting miRNA mimic in cultured cells is that they can produce excessive miRNA concentrations that are far greater than the physiological concentrations found in biological settings. As a result, changes in cell physiology or morphology due miRNA transfection may be different from the effects caused by an endogenous miRNA.

**Response:**

Thanks for your valued comment. I do agree that changes in cell physiology or morphology due to miRNA transfection are different from the real effects caused by the endogenous miRNA. However, in fact, in order to explore or verify the function of one gene (or protein or RNA) in cells, we usually have to overexpress or knockdown the expression of this gene artificially and
evaluate its effects on cells. In the process of experiments, we occasionally found that the expression of one miRNA or mRNA was hundreds of times higher (or lower) in cancer tissues than that in compared normal tissues, which actually reflected the real changes \textit{in vivo}. Sometimes, one miRNA concentration in tissue of one patient might be far greater than that of another patient. Thus, although the effects changes due to miRNA transfection couldn’t fully stand for the real biological alteration caused by endogenous miRNA, we still believe that transfection is a recommended method to evaluate the changes of effects on cell biological behavior.

Thank you very much for all of your kind patient and consideration.

Best regards

Sincerely yours,

Shan Wang