Author's response to reviews

Title: MiR-378 is an independent prognostic factor and inhibits cell growth and invasion in colorectal cancer

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Author's response to reviews: see over
Dear editor,

Thank you very much for your letter and the comments from the referees about our paper submitted to BMC Cancer (Manuscript ID 1606844959103143). The relevant regulations had been made in the original manuscript according to the comments of reviewers, and the revised portions were marked in red bold. We also responded point by point to each reviewer comments as listed below, along with a clear indication of the location of the revision.

If you have any question about this paper, please don’t hesitate to let me know.

Sincerely yours,

Dr. Guangjun Zhang

December 29, 2013

The list of comments of reviewers and the response to reviewers:

Reviewer number: 1

Reviewer's report:
Zhang et al. for the first time investigated the role of miR-378 in colorectal carcinoma and found that that miR-378 significantly down-regulated in CRC cancer tissues and cell lines. Moreover, patients with low miR-378 expression had significantly poorer overall survival as an independent prognostic factor in CRC. Over-expression of miR-378 promoted SW620 cell growth and invasion, and resulted in down-regulation of vimentin expression. However, miR-378 knock-down inhibited these processes and enhanced the expression of vimentin. In addition, we further identified vimentin as the
functional downstream target of miR-378 by directly targeting the 3′-UTR of vimentin. It was concluded that miR-378 may function as a tumor suppressor and plays an important role in inhibiting tumor growth and invasion. The paper is well organized and written, which determines it available to publish in BMC Cancer. However, the minor revision should be performed:

Major Essential Revision

No

Minor Essential Revisions

1) In the article, tissue experiment is firstly described, followed by cell and animal experiment. In contrast, the sequence for the paper title is opposite to the context.

Response to Reviewer: We have rearranged the sequence for the paper title in the revised manuscript as follows:

MiR-378 is an independent prognostic factor and inhibits cell growth and invasion in colorectal cancer

2) Why not choose the CCD-18co cell to knockdown miR-378. Is the normal cell immortalized using SV40 T antigen or something?

Response to Reviewer: The reviewer’s comments are interesting and challengeable. In this study, we only investigated the effects of miR-378 on CRC cells. We did not explore if miR-378 can make the normal colon epithelium cell (CCD-18co cell) acquire the characteristics of CRC cells. We did not read the relevant reports about the regulatory roles of
microRNA on normal colon cell lines. This may be a promising new direction for research.

There have been some attempts to immortalize or transform intestinal epithelial cells by using known immortalizing viruses or oncogenes. The immortalization of cells from the human normal colon by simian virus 40 (SV40) has been described [1]. A fetal rat normal intestinal line was established by immortalization with the human adenovirus type 2 early region E1A and polyoma virus and simian virus 40 large T tumor antigens [2]. In some studies, a temperature-sensitive mutation of SV40 large T antigen has been used to immortalize cells [3,4]. And, this temperature-sensitive mutation of SV40 large T antigen (tsA58 mutant) has been used to establish conditionally immortalized epithelial cell lines from both normal colon and small intestine of adult transgenic mice [5]. So, the normal cell can be immortalized using SV40 T antigen or something.


3) Please give the reason using SW620 cell for invasive or migrating study. Why not select SW480?

Response to Reviewer: We have two reasons using SW620 cell for invasive or migrating study.

Firstly, we analyzed the expression level of miR-378 in a panel of CRC cell lines with different degrees of differentiation and metastatic ability including HT-29 (high differentiation), HCT-116 (low differentiation), SW480 (low metastatic ability), SW620 (high metastatic ability). We observed that miR-378 expression was relatively lower in SW620 cells than in SW480 cells, HCT-116 cells and HT-29 cells (Figure 1), suggesting that miR-378 expression may be associated with the degree of CRC cell differentiation and metastatic ability. Based on this expression pattern, we therefore chose SW620 cells for the following studies.

Secondly, in the Discussion part of our manuscript, we have explained the reason as follows: A recent report showed that vimentin was one of the predominant overexpressed proteins in the highly metastatic cell line SW620 [27]. Thus, in the present study, SW620 cells were selected as model systems for the study of the molecular events involved in CRC metastasis.
4) Regarding xenograft experiment, please provide an explanation why not to perform the anti-miR-378 suppression.

**Response to Reviewer:** Due to short time and experimental funds, we did not perform the anti-miR-378 suppression. We agreed that the xenograft experiment we performed was incomplete. But, we have examined the role of pre-miR-378 on xenograft tumor, which could explain its inhibitory roles in tumor growth combined with MTT assay. In the future, we will do more research work to study its regulatory roles in CRC.

5) In Table 1, “Histology grade” should be “histological grading”?

**Response to Reviewer:** According to the reviewer's advice, we have revised the sentence in the revised manuscript, which may be more accurate.

6) As for clinical stage, I think that it should be “clinicopathological staging”. Please confirm it!

**Response to Reviewer:** We very much appreciated the careful reading of our paper. We confirm “clinical stage” should be “clinicopathological staging”, and we have revised it in the revised manuscript.
Reviewer number: 2

Reviewer's report:

The author’s study showed the inhibitory effect of MiR-378 on the cell growth and invasion of colorectal cancer cells. The results are interesting; but there are some questions need to be clarified prior to publication.

Major Compulsory Revisions

1. There are 2 mis-descriptions in abstract part need to be corrected.

In the result part of abstract, there is a description about the effect of miR-378 “Over-expression of miR-378 promoted SW620 cell growth and invasion, and resulted in down-regulation of vimentin expression. However, miR-378 knock-down inhibited these processes and enhanced the expression of vimentin.’ According to your figs and results description, I think that the right description should be “Over-expression of miR-378 inhibited SW620 cell growth and invasion, and resulted in down-regulation of vimentin expression. However, miR-378 knock-down promoted these processes and enhanced the expression of vimentin.’

Response to Reviewer: The reviewer is quite right, we made some mistakes in writing the abstract part. According to the reviewer's advice, we have revised it in the manuscript as follows:

Over-expression of miR-378 inhibited SW620 cell growth and invasion, and resulted in down-regulation of vimentin expression. However, miR-378 knock-down promoted these processes and enhanced the
expression of vimentin.

2. The manuscript structure should be re-organized. The figure legends should just follow the reference part, not below each figure.

**Response to Reviewer:** According to the reviewer's advice, the manuscript structure has been re-organized, and the figure legends have followed the reference part in the revised manuscript.

3. In the methods, it showed that there are five mice per group in the ‘In vivo xenograft experiments’, but in the results, there are only 3 tumors’ photos were shown (figure 4). Please give an explanation.

**Response to Reviewer:** When taking photographs, we did not place all the tumors on a piece of gauze. So, only 3 tumors’ photos were shown in figure 4. We have added another two tumors’ photos in the revised manuscript.

4. In your PDF file, the fig part were duplicated, please delete the repeats.

**Response to Reviewer:** According to the reviewer's advice, we have revised the fig part, and deleted the repeats in the revised manuscript.

**Figure 1** The relative expression levels of miR-378 in CRC tissues and cell lines. (A)

The relative expression of miR-378 between CRC tissues (T) and adjacent normal mucosa
The bars in the figure indicate the means of the relative expressions of miR-378. (B) The relative expression of miR-378 in CRC cell lines SW620, SW480, HCT116, HT29 and the normal colon epithelium cell line CCD-18Co. **$P<0.01$. 

**Figure 2** Kaplan–Meier survival curves of patients with colorectal cancer based on miR-378 expression status. Patients in the low expression group had significantly poorer prognosis than those in high expression group ($P=0.004$, log-rank test).

**Figure 3** Effects of miR-378 on proliferation and invasion of SW620 cell line. (A) (B) Over-expression of miR-378 inhibited SW620 cell growth and invasion. (C) (D) Down-regulation of miR-378 promoted SW620 cell growth and invasion. *$P<0.05$, **$P<0.01$. 

**Figure 4** MiR-378 inhibits tumor growth in vivo. (A) Representative image of tumors formed. (B) Growth curve drawn by measuring tumor volumes at the indicated times. (C) Weight of xenograft tumors. **$P<0.01$. 

**Figure 5** Vimentin is a direct target of miR-378. (A) The wild-type (WT) and mutated (MUT) 3’UTR of vimentin, with the seed region and base substitutions (bold). (B) The expression levels of vimentin mRNA and protein were detected by qRT-PCR and western blot assays. (C) Ectopic miR-378 expression inhibits wild-type but not mutant vimentin 3’UTR reporter activity. * $P<0.05$, **$P<0.01$. 

**Discretionary Revisions**

For the reference citation, there are another 3 papers about the expression of miR-378 in colorectal cancer tissues. I think you should include these papers in your reference.

**Response to Reviewer:** We very much appreciated the careful reading of our paper. We have added it in the revised manuscript as follows:
Finally, we are very sorry for missing an experimental method (MTT assay) in the part of the Materials and methods. We have added it in the revised manuscript as follows:

**MTT assay**

A total of $2 \times 10^4$ SW620 cells were plated onto 96-well plates for 24h. The cells were then transfected with 50 nM the indicated miRNA. At different time points (24 h, 48 h and 72 h), the culture medium was removed and replaced with culture medium containing 10µl of sterile MTT dye (5 mg/ml). After incubation at 37°C for 4 h, the MTT solution was removed, and 150µl dimethyl sulfoxide (DMSO) was added to each well followed by measuring the absorbance at 570 nm on an enzyme immunoassay analyzer (Bio-Rad, Hercules, CA, USA).