Author's response to reviews

Title: MiR-133b is frequently decreased in gastric cancer and its overexpression reduces the metastatic potential of gastric cancer cells

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Author's response to reviews: see over
Dear Ms. Cherry Battad:

We resubmit our manuscript entitled "MiR-133b is frequently decreased in gastric cancer and its overexpression reduces the metastatic potential of gastric cancer cells" to BMC Cancer for publication.

The old manuscript ID: 1908295334100373.

In this report, we found that expression of miR-133b was significantly down-regulated in 70% (98/140) of the gastric cancer tissues compared with adjacent non-tumor tissues. Expression of miR-133b negatively correlated with lymph node metastasis of gastric cancer in patients. Similarly, the expression of miR-133b was much less in seven tested gastric cancer cell lines than in a immortalized gastric epithelial cell line GES-1. Overexpression of miR-133b significantly inhibits migration and invasion of gastric cancer cells in vitro. In a mouse gastric cancer model, overexpression of miR-133b suppressed peritoneal spreading of metastasis. Moreover, the transcriptional factor Gli1 was identified as a direct target for miR-133b. Subsequently, Gli1 target genes OPN and Zeb2 were also inhibited by miR133b. These results suggested that miR-133b plays an important role in gastric cancer metastasis.

The work described has not been submitted elsewhere for publication, in whole or in part, and all the authors listed have seen the manuscript and approved to submit to your journal.

The authors have declared that no competing interests exist.

Thank you very much for your attention and consideration.

Thank you for your kind review of our manuscript. According to your suggestions, the revised manuscript has been submitted. Details changes made in response to reviewers' comments are addressed point by point as follows:

**Responses to Reviewers:**
Reviewer: Gennaro Colella

Reviewer's report:

Minor Essential Revisions

1) Methods/Cell migration and invasion assays, lane 1: replace “16h” with “16 h”;

Answer: We are sorry for having made this error. We have corrected it.

2) Methods/retroviral transfection for stable cell lines, lane 1: what does it mean “…retrovirus containing miR-133b or on insert…”? Probably did you mean “…retrovirus containing miR-133b or no insert…”? If so, please correct;

Answer: Yes. We have corrected it.

3) Methods/statistical analysis, lane 5: replace “means” with “mean”;

Answer: Done.

4) Results/The expression of miR-133b is down-regulated in GC, lane 2: replace “GC[6]” with “GC [6]”;

Answer: Done.

5) Results/Gli1 is a target gene of miR-133, lane 3: replace “miRanda[26], TargetScan [27] and RNAhybrid[28]” with “miRanda [26], TargetScan [27] and RNAhybrid [28]”;

Answer: Done.

6) Discussion: carefully check for the presence of spaces between word and reference (e.g.: lane 2, “Fugu[37]” must be “Fugu [37]”) along the text;

Answer: We are sorry for having made those errors. We have checked and corrected them.

7) Figure legends/Figure 1, lane 1: replace “there” with “their”;

Answer: Done.

8) Figure legends/Figure 3, lane 3: replace “means” with “mean”;

Answer: Done.

9) Figure legends/Figure 4, lane 6: replace “means” with “mean”;

Answer: Done.
10) Additional files/Additional file 1 legend, lane 2: replace “means” with “mean”;

Answer: Done.

Reviewer: Manuela Garibaldi

Reviewer's report:

Major Compulsory Revisions:

1 in answer to the question:” In fig 4C reduction of GLI1 expression after introduction of miR-133b is detectable, I have some concerns about reduction of the two genes that are target of GLI1 (ZEB2 and OPN)”, authors added results of mRNA expression for ZEB2 and OPN that show a reduction of the two genes in presence of miR-133b mimic. This result does not support a reduction of the two genes at protein level, thus the sentence stating that "protein levels of ZEB2 and OPN were markedly reduced in MKN-28 cells transfected with miR-133b mimic" must be deleted. Authors should also discuss this finding (reduced expression of ZEB2 and OPN mRNA but not of their corresponding proteins) in the discussion.

Answer: We are sorry for not choosing clearer representative blot picture of ZEB2 and OPN. We have showed clearer blot picture (ZEB2 and OPN) and added the quantification for three independent blots in our new manuscript (Figur 4D).

1 The authors did not reply to the query regarding the association between miR-133b expression and clinical features of GC. In the answer they explained the meaning of the variable used for stratification of samples but not explained how they chose the cut off (<0.5, 0.5-2.0 and >2). As mentioned in the review, how this choice was made is very important for validity of the results: "The criteria used for dividing cases into three groups according to expression of miR-133b are not indicated. Was the choice of the cut-off values based on
previous knowledge? If the authors chose the cut-off based on the observed association between the expression of the miR and the outcome, the reported results would not be valid (overestimation of the real association). A better categorization should be obtained by determining cut-off values using tertiles.

Alternatively, the association between the expression of the miRNA and the clinical characteristics of cases must be done using miRNA levels as continuous variable. This type of analysis would guarantee a better statistical power compared to the analysis that uses the categorized values of the miR”.

Please explain the choice or repeat the analysis as previously suggested.

Answer: Thanks for the excellent suggestion. We are sorry we didn’t make it clear in our former cover letter. The choice of the cut-off values was based on previous knowledge. Fold change=2 or 0.5 was considered to statistically significant difference [1, 2]. Suppose all the value of fold change were >2, all the cases would be on the “high group”, but categorization by determining cut-off values using tertiles would still put 25% cases into “high group”.

Each case had two data, expression of miR-133b in cancer tissue and expression of miR-133b in adjacent non-tumor tissues. We had miR-133b levels as continuous variable in Figure 1B. We can use miR-133b levels of in cancer tissue alone or miR-133b levels of in adjacent non-tumor tissues alone as continuous variable to analysis the association between the expression of the miRNA and the clinical characteristics of cases. But in this way, we will miss the information of miR-133b expression in another tissue in the same case.

1 Again, English must be edited.

Answer:
Point is well taken. English editing has been made as advised. We now have corrected the typos and a number of grammar inconsistencies in the revised manuscript.
Sincerely yours,

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