Reviewer's report

**Title:** MiR-133b is frequently decreased in gastric cancer and inhibits cell metastasis

**Version:** 2  **Date:** 8 July 2013

**Reviewer:** Gennaro Colella

**Reviewer's report:**

Minor Essential Revisions

1) Please carefully read the manuscripts and correct typing errors such as lack of space and/or double space between two words (e.g. in Background section at lane 14 where a space between “tissues” and “[6] must be introduced”)

2) Please carefully read the manuscript and replace “mimics” with “mimic” when you are talking about a single miRNA mimic (e.g miR-133b mimic, NC mimic);

3) Please carefully read the manuscript and replace all “P<0.XXXXX” with “p < 0.XXXX” to uniform them;

4) In my opinion the TITLE is misleading because it seems that in gastric cancer the repression of cell metastasis is positively correlated to a lower expression of miR-133b. I think that a title like “Mir-133b is frequently decreased in gastric cancer and its overexpression reduces the metastatic potential of gastric cancer cells” sounds better;

5) “Background” section, lane 24: replace the phrase “…that miR-133b could repress…” with “…that miR-133b overexpression could repress…”;

6) “Methods/Cell lines and culture” section, lane 3: replace “MKN45 and MKN28” with “MKN-45 and MKN-28”;

7) “Methods/RNA Isolation and…..” section, lane 1: add the “CA,” after “Carlsbad,”;

8) “Methods/RNA Isolation and…..” section, lane 4: replace “Ferments” with “Fermentas”;

9) “Methods/RNA Isolation and…..” section, lane 6: add City, State and Country where Applied Biosystems is;

10) “Methods/RNA Isolation and…..” section, lane 7 and 17: in “DDCt” replace DD with greek characters;

11) “Methods/Transient transfection…..” section, lane 2: add space between “(NC)” and “(Sense:..)”;

12) “Methods/Transient transfection…..” section, lane 5: replace “Lipofectamine2000TM” with “LipofectamineTM 2000” and delete “Carlsbad, CA, USA”;

13) “Methods/Cell migration and…..” section, lane 2/3: if it is not necessary to
indicate the Catalog number of the product, please delete it. Add the City and the State where BD Discovery Labware is;

14) “Methods/Cell migration and….” section, lane 8: add “, USA” after “MA”;
15) “Methods/Construction of the reporter….” section, lane 12: delete “, USA” if the Promega distributor of the Dual-Glo Luciferase assay and pRL-TK vector is the same;
16) “Methods/Western blot analysis” section: please indicate City, State and Country of the distributors of the antibodies used;
17) “Methods/Retrovirus production” section: please re-edit the text of the section for a more clear comprehension;
18) “Methods/Statistical analysis…” section, lane 5: replace “Means±SDs” with “Mean±SD”;
19) “Methods/Statistical analysis…” section, lane 6: add City and State where IBM is;
20) “Results/Overexpression of … in vitro” section, lane 11: replace “metrix gel” with “matrix gel”;
21) “Results/Gli1 is…. ” section, lane 2: replace “algorithms software” with “algorithm softwares”;
22) “Results/Gli1 is…. ” section, lane 8: replace “was” with “were”;
23) “Results/Gli1 is…. ” section, lanes 9 to 12: please re-edit the text for a more clear comprehension;
24) “Results/Gli1 is…. ” section, lane 21: add “mimic” after miR-133b;
25) “Figures/Figure1” section, lane 1/2: replace “its” with “their” and check for character dimension uniformity;
26) “Legend to Figures/Figure1” section, lane 7: replace “means” with “mean”;
27) “Legend to Figures Figures/Figure2” section, lane 4: replace “means” with “mean”;
28) “Legend to Figures Figures/Figure3 (B)” section: in my opinion it should be better to put a phrase like “Quantification of the peritoneal nodules is shown in the bar graph. The results are the mean of 6-10 mice ± SD” rather than a description of the results;
29) “Legend to Figures Figures/Figure4 (B), (C), (D)” section: it should be better to describe what graphs and/or blots show rather than comment the results and/or shortly describe the method…;
30) “Table1” section: it should be useful to have an exhaustive legend for the Table1;
31) “Additional files/additional file1 Legend” section: the title of the legend cannot be part of the legend text. Please correct it.
32) Figure 1B: the graduation of the y axis of the graph is not clear. Why 0.3 is located in the top segment and not in the bottom segment? I imagine that 0.3 is the last (higher) value of the bottom segment whereas 1 can be the first (lower)
value of the top segment. If so, please correct it.

33) Figure 2: in all the panels (both pics and graphs) please replace “NC” and “miR-133b” with “NC mimic” and “miR-133b mimic”;

34) Figure 3B: to uniform labels of panels A and B of the figure replace “NC” and “miR-133b” with “RV-miR-NC” and “RV-miR-133b” respectively in the x axis of the graph in panel B;

35) Figure 4: in all the panels (both pics and graphs) please replace “NC” and “miR-133b” with “NC mimic” and “miR-133b mimic”;

36) Supplementary Figure S1 and S2: in all the panels (both pics and graphs) please replace “NC” and “miR-133b” with “NC mimic” and “miR-133b mimic”;

Major Compulsory Revisions

1) “Methods/Transient transfection of miRNA mimics” section: the negative control mimics are two single stranded oligonucleotide or, as I imagine, the two strands of a double stranded oligonucleotide which is generated after an annealing procedure? If the latter is the case I was not able to find complementarity between sense and antisense strand. Could you clarify this point?

2) “Results/The expression of…” section, lanes 7 and 8: here the authors say that “expression of miR-133b was much less in all eight GC cell lines tested” but on the basis of the data reported in figure 1C at least one cell line, BGC-823, shows miR-133b levels quite similar to those found in GES-1, Could the authors comment it?

3) “Table 1” section: in the expression category “Low” (n = 67) the sum of patients with > 60 years (36) and ≤ 60 years (29) is 65 and not 67: why?

4) “Table 1” section: in all the expression categories the sum of patients with Distal/Middle/Proximal third Location is different from 67, 55 and 18: why?

5) The authors suggest that “miR-133b-Gli1-Zeb2/OPN” pathway seems to be important in reducing the metastatic potential of gastric cancer cells. Like Gli1 also Zeb2 and OPN are important effectors of this pathway thus, in my opinion, in order to have a sort of double check and a more complete picture of the molecular status of the three protein players of the pathway it should be useful to confirm the Gli1-mediated downregulation of Zeb2 and OPN protein expression also at mRNA level by performing a qRT-PCR assay;

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests