Reviewer’s report

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

Version: 2 Date: 3 July 2013

Reviewer: manuela gariboldi

Reviewer’s report:

This work shows that miR-133b, which is downregulated in gastric cancers with lymph node metastases, can inhibit metastases both in vitro and in vivo. This mechanism probably involves Gli1, a transcription factor directly correlated with lymph node metastasis in gastric cancer that the authors demonstrated to be a direct target of miR-133b.

- Major Compulsory Revisions

In vitro and in vivo data regarding the role of miR-133b in inhibiting metastases are new. It is not clear the reason for they decided to investigate miR-133b, among the 15 miRNAs they selected in the paper they cite (ref 6).

The association between reduced miR-133b expression and presence of lymph node metastases has already been found by Wu WY et al., (Wu WY, Xue XY, Chen ZJ, Han SL, Huang YP, Zhang LF, Zhu GB, Shen X. Potentially predictive microRNAs of gastric cancer with metastasis to lymph node. World J Gastroenterol. 2011; 17:3645-51. PMID: 21987613).

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.

In the version of miRanda available at www.microrna.org (Aug 2010) no prediction for miR-133b targeting GLI1 is present. Authors should explain how they identified the putative interaction between miR-133b and GLI1. Was it retrieved in an older version of the program? If so, specify which.

Add references for the two prediction databases.

In the section of “In vivo metastasis peritoneal spreading assay” indicate the cell line used for the experiment.

Were cells tested and authenticated?

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).

The discussion section is very poor. Authors should describe the reason for they selected GLI1 among the putative targets of miR-133b. There are other miR-133b targets that are involved in metastasis development and spread, for example one of them is the MET oncogene. A description of the known functions of GLI1 and of the possible related effects on metastasis development should be also added.

The English is poor, the manuscript needs a strong revision.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests