Reviewer’s report

Title: Silencing microRNA-330-5p increases MMP1 expression and promotes an invasive phenotype in oesophageal adenocarcinoma

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Reviewer: ARMANDO FELSANI

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Bibby et al., Silencing microRNA-330-5p increases MMP1 expression and promotes an invasive phenotype in oesophageal adenocarcinoma

In this manuscript, the authors identify by RNA-seq the genes whose expression is up- or down-modulated by silencing miR-330-5p in an oesophageal adenocarcinoma (OEC) cell line. They found 8 genes upregulated and 34 genes down-regulated. Then they investigated the effect of miR-330-5p suppression on OEC biology.

My first concern about this paper, potentially quite interesting, is that the authors first declared that they would investigate the role of miR-330-5p downregulation on the invasive phenotype of OEC, but then they focused themselves just on one of these genes, MMP1, excluding all the others from further studies. This decision, really not justified in my opinion, greatly limits the importance and the conclusions of the work in question.

To identify the effects of miR-330-5p silencing, the authors correctly established two stable OE33 miRZIP-330-5p model cell lines, SC and HC. It is not clear if the two-model strategy was also adopted for the control cells. The control cells used in the experiments with miRZIP-330-5p SC were the miRZIP-VC SC cells, but this cell line was not described in Materials and Methods and poorly defined in the Results. On the other side, it is not clear which control cells were used in the experiments with miRZIP-330-5p HC: did the authors use the miRZIP-VC SC cells again or matching miRZIP-VC HC cells?

Another general criticism is about the criteria of use of the two-model strategy, since the authors performed the experiments either on one or the other model or both, often without convincing explanations.

In conclusion, in my opinion this manuscript could be much more worthy of publication if the described experiments, started with an unbiased screening, had been conducted with a more systematic approach and with a more comprehensive view of the biology of this tumor, without focusing in an unjustified way on a single aspect.
Specific points:
Why the PCR quantitation experiments (Figure 1) performed in order to validate the sequencing data were made only with the SC cells, that were those used in RNA-seq experiments, and not also with the HC ones, that have been used in most of the subsequent experiments?

Figure 2. The authors should use an independent protein loading control, for all the figure panels, also if they assume that MMP7 protein does not change its expression in the two cell lines. Moreover, since the data about MMP7 in panel (A) are not shown and there is not a loading control, I wonder how they demonstrated that MMP7 protein expression did not change.

It is puzzling the behavior of MMP7 upon miR-330-5p silencing, it increased at the RNA level but remained unchanged at protein level in the conditioned medium. I think it could be useful to measure the MMP7 protein level inside the cell because this behavior could be due to a processing/exporting limiting mechanism.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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I am able to assess the statistics

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