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

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Reviewer: Safiye Nese Atabey

Major concerns:
1. Validation studies to proof MMP1 as a target of miR-330-5p are not sufficient.
   a. As published elsewhere, neither computational analysis nor qPCR results prove that MMP1 is a target molecule for miR-330-5p. To determine whether or not the predicted sites for miR-330-5p in the 3'UTR MMP1 is responsible for MMP1 down regulation, more experiments should be performed, such as; luciferase reporter assays with vectors containing the wild type MMP1 3'-UTR and the mutant MMP1 3'UTR.
   b. Authors hypothesized that miR-330-5p likely directly targets the MMP1 mRNA and they transiently overexpressed miR-330 in the OE33 cell line to prove MMP1 as a target mRNA for miRNA-330-5p. Clarification of the clones (SC or HC) and miR vector used for this experiment needs to be clarified.
2. It would be helpful if authors explain why they use heteroclones in some experiments whereas the used monoclones in other experiments. Is there any specific reason? For instance, Matrigel-based transwell invasion assay was performed using HC, however SC cells was used for collagen transwell invasion assay. Did author use perform those experiment using both clones? Did they get similar results? HC did not display a more invasive phenotype at the time points tested in the Matrigel-based transwell invasion assay.
3. The in vivo experimental model is not compatible with the main objective of the study. This study mostly focused on the role of miR-330-5p expression level on the invasion capacity of esophageal adenocarcinoma, whereas in vivo experiments were designed to test tumorigenic potential...
of miR-330-5p. In vivo data presented in the manuscript showed that OE33 miRZIP-330-5p HC xenografts grew significantly faster than the miRZIP-VC HC xenografts. There were no data presented on the neither invasive character of these xenografts, or MMP1 expression levels etc. In addition the differential effects of collagen and Matrigel on tumor growth and invasion were not tested in this xenograft model.

4. The key point of the study is the regulatory role of miR-330-5p on MMP1 expression and their involvement in OAC tumours. Thus it is essential to demonstrate expression pattern of these molecules in the sample patient samples.

5. Authors suggested that loss of miR-330-5p expression in OAC may influence tumour cell invasive capacity, tumour growth and therapeutic sensitivity via alterations to the tumour microenvironment. However, the study does not contain any study demonstrating that miR-330-5p could possibly increase therapeutic sensitivity. Particularly in the line 106 authors suggest that in the study the implications of miR-330-5p downregulation in OAC neo-CRT non-responders were further investigated. However, there is no data related with neo-CRT non-responders.

Minor concerns:

1. Authors mentioned that OE33 are considered poorly invasive in cross-linked collagen, however, it has been shown that collagen stiffness is associated with tumour progression in esophageal squamous cell carcinoma. Several studies have shown that increased expression of LOXL2 which is a collagen cross-linking enzyme, enhance proliferation, migration and invasion in esophageal squamous cell carcinoma (1). Indeed a positive correlation has been shown in the expression of LOXL2 and MMP1 in cancer (3) expression. Could authors discuss composition and role of ECM in the progression of this cancer (particularly involvement of cross-linked collagen) since invasion results were different in Matrigel assay than in collagen assay.

2. Different styles of referencing have been used in the manuscript. Authors need to follow one style while preparing reference list.

References


2. Ying Zhu MS, SMYD3 stimulates EZR and LOXL2 transcription to enhance proliferation, migration, and invasion in esophageal squamous cell carcinoma. Volume 52, June 2016, Pages 153-163


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

No

Does the work include the necessary controls?
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No
Are the conclusions drawn adequately supported by the data shown?
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