Reviewer's report

Title: miR-125b induces cellular senescence in malignant melanoma

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Reviewer: Soheil S.S Dadras

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In this study Nyholm et al. has investigated the mechanism of miR-125b as a continuation of their prior findings on this miRNA being down-regulated in melanoma. The authors demonstrated the expression of miR-125b by ISH and attempted to dissect the molecular mechanism of action of miR125b in vitro and in vivo (xenotransplants) using one cell lines. Although, this is an important area of study and the authors have some convincing preliminary data, there are some major deficiencies as enumerated below.

Major:
1. In general, the data is very descriptive and in many places the results need to be quantified.
2. Need to specific how many cases were tested for miR125b expression by ISH. There is no quantification of this data and it is unclear whether miR-125b is increased in nodal metastasis than the primary lesion in figure 1a and b. explain the location of ISH signal. Cytoplasmic or nuclear?
3. What is the colony count for colony formation assay? Quantification?
4. Unclear what the molecular findings mean in the transfected cells, up-regulation of P27, P53 and P21 mediated by miR-125b
5. Could the ISH results be confirmed by qRT-PCR in human tumor samples?
6. The authors made definitive statements that miR-125b decreases amount of proliferation by measuring ki67 and cyclin D1. However, some cell based assay to measure the proliferation and invasion of the Mel-Juso cells when they are transfected by miRVec-125b and miRVec-control.
7. Although this study was on miR-125b as a senescence inducer in malignant melanoma, in ISH assay for miR-125b, it is recommended to have nevus slides/samples from the patients as a control along with malignant melanomas, lymph node metastases, and superficial spreading MM.
8. The introduction is missing some important description of latest miRNA characterization esp. by next generation sequencing in melanoma and other studies on miR-125, see below:


9. Since the authors showed only one image from their miR-125b ISH on human FFPEs. It is recommended to quantify the ISH results and be presented as a table/graph added to manuscript.

10. In figure 3 authors, have put both ISH and IHC results together. It is recommended to either separate the results or explain the methodology in figure legend as Ki67 and cycline D expression was visually quantified using IHC.

Minor:
1. Label the figures more clearly and appropriately
2. Page 9 last paragraph and last 2 lines: “Lungs and liver were taken out and cut in to three parts each, which were treated as the tumour samples”. Does author means these organs were checked for distance metastases?

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: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests