Nyholm et al investigated the role of microRNA-125b in melanoma senescence. The authors observed variability of expression of miR-125b in primary and metastatic melanomas indicating that miR-125b may be expressed at a higher level in cells showing signs of senescence. Ectopic overexpression of miR-125 in a melanoma cell line resulted in the induction of several markers of senescence as well as decreased growth. Overexpression of miR-125b in tumor xenograft experiments revealed that miR-125b suppressed markers of cell proliferation and induced markers of senescence without effect on tumor mass.

Comments:

1. The observation about the relation of miR-125b expression to cell size is not entirely clear. First, most of the ISH-signal for miR-125b expression (Figure 1) seems to be localized in the cell nuclei rather than in the cytoplasm as it would be expected for a miRNA. How do the authors interpret this? Was the ISH signal specific in this experiment? U6 staining is shown as positive control on healthy skin, but not that of miR-125b. The staining should be performed on the same tissues both for the miRNA and for the controls.

2. The staining intensity for miR-125b was very heterogeneous both in primary melanoma samples and in lymph node metastases. Unfortunately, the arrows on the figure do not clearly point to cells with particularly high miR-125b expression. Furthermore even cells with small cytoplasm expressed miR-125b at a high level. Cell size is difficult to assess without counterstain, which is difficult with ISHs. The authors could complement the figure with HE-staining on serial sections. Alternatively the authors could consider removing Figure 1 from the revised version of the manuscript, because it is not absolutely necessary for drawing the conclusion about the effect of miR-125b on melanoma cells.

3. What was the transfection efficiency like in the miR-125b-overexpression studies?

4. Please show standard deviation or standard error of mean for the results of the colony formation and proliferation assays obtained with the miR-125b-overexpressing cells (Figure 2). If Figures would become too large, the authors could consider dividing it into two.

5. The in situ hybridization results obtained with the mouse xenograft tumors reveal a different staining pattern as compared to that with the human tumors. In
this case the staining is cytoplasmic, as expected. However, miR-125b does not seem to be detectable in the control tumors in contrast to human tumors shown on Figure 1. What is the explanation for this?

6. The ISH-picture of the control tumor on Figure 3A is completely blank. Tumor structure is not visible. Consider complementing this figure with HE-staining on parallel sections.

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

Quality of written English: Acceptable

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