Reviewer's report

Title: Dysregulated miR-183 Inhibits Migration in Breast Cancer

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Reviewer: Aaron Sarver

Reviewer's report:

The authors describe the levels of miR-183 in 70 tumor tissues for which clinical data was available and in breast cancer derived cell lines and show minor differences between subtypes. The authors show that over-expression of miR-183 in T47D leads to decreased migration in T47D but not in other cell lines. The authors show that changes in gene expression occur following overexpression of miR-183 using a gene panel and that qPCR shows similar results. The authors go on to show Immunocytochemistry for VIL2 following miR-183 over-expression.

Differential expression of miRNA in breast cancer subtypes is very interesting but the reported levels of change appear to be minor. The interaction between miR-183 and VIL2 is interesting and has been previously characterized for lung cancer (2008).

Major Compulsory Revisions

1) The fold changes in miR-183 observed in the different intrinsic subtypes of tumor tissues appear to be very modest (less than 1.5 fold average change) and the average fold change between groups is not reported anywhere. This is essential to determine whether or not "Statistically significant" changes are diagnostically useful and is currently not reported in the text. Gene profiling experiments usually disregard miRNA with fold changes less than a certain threshold.

2) The authors used a type of normalization in their RQ-PCR normalization that is non standard for miRNA measurement. For example, U6 is commonly used for normalization (Cancer Res. 2005 Aug 15;65(16):7065-70.) The authors do not describe why they deviate from standard U6 based normalization for RQ-PCR. A possible interpretation of the data is that the normalization method used (normalization to Let7a and miR-16) may be leading to the changes observed. In reference 12 Figure 5 and 6 Let7A is shown to have differential expression in breast tumors dependent on clinical features and appears inappropriate as a control.

3) The current title "Dysregulated miR-183 Inhibits Migration in Breast Cancer" is only supported by data in T47D cells and is NOT supported in SKBR-3 and MDA-MB-231 cells thus the use of the term Breast Cancer is inappropriate. A more accurate title would be 'Overexpression of miR-183 in T47D cells decreases in vitro migration'. This comment also applies to results header on
3) The work surrounding VIL2 presents more questions then answers. Does miR-183 overexpression lead to decrease in VIL2 protein levels in T47D cells as measured by western blot? What happens in the other cell lines?

Minor essential revisions
1) The model for miR-183 mediated inhibition of migration is not clear in T47D. Is it transcriptional control, translational control, localization control or a combination of the above that is hypothesized as the mechanism?

2) The statement "firstly T47D cells were shown to express the lowest endogenous levels of miR-183, thus the effect of overexpression in these cells would be expected to result in a more dramatic or measurable phenotypic effect than in cells where miR-183 is expressed at higher levels endogenously." Is misleading as MDA-MB-231 cells are shown in FIGURE 2 to have almost identical miR-183 levels as T47D.

3) The following statement is not understandable. "Downregulation of miR-183 expression was validated using RQ-PCR, indicating that regulation occurred at the mRNA level, this is in contrast to the findings of Wang et al[13] who found regulation of Ezrin to be at the posttranscriptional level in lung cancer." Possibly the authors mean Downregulation as a result of miR-183 expression?

4) In the following sentence it is unclear which SKBR-3 cells exhibited decreased migration as Figure 3 shows miR-183 overexpression leading to increased migration in SKBR.

"In the SKBR-3 cells which did exhibit decreased migration, there appeared to be decreased membranous and cytoplasmic expression of Ezrin in the transfected cells (figure 6b), this suggests that the overexpression of miR-183 in these cells had disrupted Ezrin expression but this had not translated to a measurable functional effect."

Discretionary Revisions
1) One potential way to turn this into an article of general interest would be to determine whether VIL2 decrease via rnai alone is sufficient to recapitulate the miR-183 overexpression migration phenotype in T47D cells. In light of the previously published work, presentation of this data would show a clear advance in understanding.

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, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests