Reviewer's report

Title: MicroRNA expression as risk biomarker of breast cancer metastasis: a pilot retrospective case-cohort study.

Version: 1
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Reviewer: Jeremy Squire

Reviewer's report:

Nice study with a useful discovery cohort that can be used to survey microRNAs of prognostic value for breast cancer metastasis. The use of the assay on FFPE provides some wide appeal.

The manuscript is in general well-constructed but the rationale for selecting the miRs (21, 494, and 183) are confusingly presented. Some figure elements do not contribute useful data and there is redundancy in the discussion. This provides readers with an overall lack of clarity and focus in several places.

Major

1. The three miRs were also selected because of their functional role in addition to their expression differences shown in the arrays. For example the rationale for choosing miR183 for further study has not been presented. The reason miR21 and 494 were selected from the overlapping Venn circles and the other 5 excluded also needs to be explained more clearly. The overall rationale for the study as presented in the abstract and the background is not really described in an accurate way. The selection of the three miRs was based not only on their differential expression but also on the likely function for their target pathways. This part of the study rationale was not apparent until page 13.

2. Please provide more information on the extraction of RNA from FFPE and details on the quality metrics of the templates obtained. Formalin fixation is notorious for RNA degradation, so it would be reassuring to see that the approach taken in this paper is robust. Without this information there is the concern that the results are related to differential RNA stability from fixation damage and/or the extraction procedure.

3. Figure 1 does not contribute to gene selection. It should be moved to supplementary but it would be helpful to arrow the three selected miRs (183, 494 and 21) so that readers can easily see how well expression levels are associated with the phenotypes.

4. The discussion is too general in many places. Please focus on the findings from the study and the potential future directions.

Minor

1. The probes table 2 can be moved to supplementary.
2. Please briefly explain use of ROC curves for more general readership.

**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.