Author's response to reviews

Title: Dysregulated miR-183 Inhibits Migration in Breast Cancer

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Author's response to reviews: see over
Dear Dr. Norton,

We recently submitted the above manuscript for publication in your journal and revisions were requested following review and editorial discussion. The reviewers concerns are addressed below:

**Response to Reviewer 1 – Aaron L**

**Compulsory Revisions**

1. *I agree that the authors have shown that their data is statistically significant. To ensure that the size of these changes is fairly represented please show dotplots representing each tumor sample value in addition to or instead of bar charts for each of the groups in figure 1. Also include the Normals and a group composed of all tumors and use the same scale for each. For an example look at figure 2 (Schetter et al. JAMA. 2008;299(4):425-436.) Additionally, make clear to all readers in the text the average difference between groups in terms of fold change.***

**Response**

Dot-plots have been generated representing each tumour sample as requested (see below). As this data is already represented in bar charts we do not feel that these dotplots add anything additional to the manuscript, however, we would be happy to include them either in the manuscript or as supplementary material if the editors feel this is appropriate.

The last figure which depicts RQ values for tumour samples compared to tumour associated normal was not included in the original manuscript as the comparison is between 70 tumours and only 9 TAN samples and thus is an unbalanced comparison.

**Figure 1 a – miR-183 expression in ER+ve tumours compared to ER-ve tumours**
Figure 1 c – miR-183 expression in HER2/neu +ve tumours compared to HER2/neu –ve tumours

Figure 1-d miR-183 expression in breast cancer subtypes
With regard to the average difference between groups in terms of fold change, this has been included in the text in the results section where the RQ-values are included in brackets for ER +ve vs ER-ve and HER2/neu+ve vs HER2/neu –ve tumours. The RQ value here has been generated using the $2^{-\Delta\Delta CT}$ equation which determines fold changes in samples relative to the lowest expressed sample. Thus the difference in average fold change between groups is included in the text.

2. The authors need to address this comment: “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” or to restate the observed changes could be occurring due to the use of let7A in the normalization. To ensure that this is not occurring determine that miR-16, when normalized using only Let7A and the delta delta method does not show statistically significant differential expression in this dataset grouped as described in the paper.

Response
As suggested, miR-16 expression was determined in each sample using only Let7A as the endogenous control and the delta delta CT method. This was performed using qBase software. RQ of miR-16 in breast tumour and tumour associated normal tissue (TAN) was then analysed in the dataset grouped as described in the paper.

There was no significant difference in miR-16 expression between tumour samples (RQ 17.3+/−3) and tumour associated normal (RQ 24+/−6) samples p=0.2, paired samples t-test.

Expression of miR-16 in breast tumour samples was then analysed to assess for statistically significant differential expression of miR-16 in this dataset grouped as described in the paper.

a. Grouped according to tumour ER status
There was no significant difference in miR-16 expression between ER +ve and ER – ve breast tumours

RQ miR-16 in ER +ve tumours 14.07+/−2 vs RQ miR-16 in ER –ve tumours 13.25+/−1.7 p=0.79 t-test

Log10RQ miR-16 in ER+ve tumours 0.97+/−0.05 vs log10RQ in ER-ve tumours 1.008+/−0.06, p=0.66 t-test

b. Grouped according to tumour PR status
There was no significant difference in miR-16 expression between PR+ve and PR-ve breast tumours

RQ miR-16 in PR+ve tumours 11.77+/−1.9 vs RQ miR-16 in PR-ve tumours 13.27+/−1.9. p=0.6 = t-test

Log10RQ miR-16 in PR+ve tumours 0.916+/−0.05 vs log10RQ miR-16 in PR-ve tumours 1.00+/−0.07. p=0.3, t-test

c. Grouped according to tumour Her2/neu status
There was no significant difference in miR-16 expression between HER2/neu +ve and HER2/neu –ve breast tumours

RQ miR-16 in HER2/neu +ve tumours 13.1+/−2.0 vs RQ miR-16 in HER2/neu –ve tumours 12.37+/−1.85. p=0.813, t-test

Log10RQ miR-16 in HER2/neu +ve tumours 1.03+/−0.06 vs log10RQ miR-16 in HER2/neu –ve tumours 0.93+/−0.05. p=0.27, t-test
d. Grouped according to Histological Subtype
There was no significant difference in miR-16 expression across histological breast cancer subtypes. P=0.934, ANOVA

e. Grouped according to Intrinsic Breast Cancer Subtype
There was no significant difference in miR-16 expression across the intrinsic breast cancer subtypes (LuminalA, LuminalB, HER2/neu overexpressing and Basal) p=0.994, ANOVA

f. Grouped according to Tumour Grade
There was no significant difference in miR-16 expression across tumour grades 1-3 p=0.207, ANOVA

g. Grouped according to UICC Tumour Stage
There was no significant difference across different tumour sages p=0.523, ANOVA

This confirms that *miR-16* is stably expressed across the clinical samples used in this study and that this expression is not altered by the use of let7a as an endogenous control.

This data strongly supports our belief that the observed changes in *miR-183* expression reported in this paper are not occurring due to the use of *let7a* in the normalization.

Response to Reviewer 2 – Pedro Gonzalez
There were no additional comments from Dr. Gonzalez.

Additional formatting requests:
We note that you have not included your conclusions within your manuscript. Please do insert this after your Discussion section.
A short conclusion section has been added after the discussion section.

We hope that we have addressed the reviewers concerns adequately. We feel that this article is likely to be of great interest to cancer researchers and look forward to your response to these revisions.

Yours Sincerely
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