Author's response to reviews

Title: Dysregulated miR-183 Inhibits Migration in Breast Cancer

Authors:

Aoife J Lowery (aoife.lowery@gmail.com)
Nicola Miller (nicola.miller@nuigalway.ie)
Roisin M Dwyer (roisin.dwyer@nuigalway.ie)
Michael J Kerin (michael.kerin@nuigalway.ie)

Version: 4 Date: 9 September 2010

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:

1. “It would be useful to the reader for the authors to provide a rational for why previous work(1) shows differential expression of Let7a in breast cancer subtypes while this work uses Let7a for normalization”

Response: The reviewer refers to work by Sempere et al (1) in which microRNA expression was determined in a number of clinical breast tumour samples and breast cancer cell lines, with a focus on miRNA expression in different cell types within the breast cancer microenvironment. However, it does not actually show differential expression of let7a in breast cancer intrinsic subtypes. In this report, let7a was shown to be widely expressed in breast tumour samples and it is shown in Figure 1b, using northern blotting, that let7a is expressed across all the tumour samples. Similarly in Figure 1c and d there is consistently high expression of let7a across all the breast cancer cell lines. This is in contrast to miR-183, miR-141, miR-200c, miR-221 and miR-222 which were expressed at significantly differing levels in luminal compared to basal breast cancer cell lines.

These authors do report a difference in let7a expression between different cell types with decreased let7a expression in cells of epithelial origin in carcinoma compared with normal, and preferential expression of let7a in luminal cells (the inner epithelial cells of the lobular alveolar unit of the breast, which are potential milk secretory cells) compared to myoepithelial cells (the outer layer of contractile cells). Thus, the differential expression is between cells within a tumour sample rather than between different breast cancer subtypes (Luminal A, Luminal B, Basal-like and HER2-overexpressing as defined by Sorlie et al (2)).

This finding indicates that miRNA profiles, and the expression of miRNA such as let7a may be altered as a result of the cellular composition of tumours; this highlights the importance of sample number in determining the range of tumour variation. To our knowledge, this is the largest cohort of breast tumours in which miR-183 expression has been analysed using RQ-PCR.
As previously discussed, we have experimentally optimized our RQ-PCR and have published results validating the use of *miR-16* and *let7a* as endogenous controls in our breast tumour samples due to their stable expression across a large range of breast tumour specimens. We have also provided evidence that the expression of *miR-16* remains stable when normalized to *let7a* alone as requested by the reviewer. We do not feel that the use of endogenous controls should be the focus of our current study relating to *miR-183*.

2. “Additionally the authors should acknowledge the mir-183 northern blots published in 2007(1) and show differential expression of miR-183”

**Response:** Sempere *et al* (1) show that miR-183 expression, as determined by Northern blot, is significantly different in breast cancer cell lines representative of the luminal subtype compared to the basal subtype. They do not report differential expression in clinical tumour samples. We have acknowledged this and referenced this report in the discussion section of the manuscript (page 14, second paragraph).

References


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
Dr. Aoife Lowery & Dr. Nicola Miller
Department of Surgery
National University of Ireland Galway
Ireland