Reviewer’s report

Title: Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma

Version: 5 Date: 26 February 2009

Reviewer: Thomas A Hughes

Reviewer’s report:

The authors have addressed most of my points nicely. However, I am still concerned by two issues relating to my original points 5 and 8/10. I think both are still major compulsory revisions – although both should be simple enough to address.

Point 5 related to the specificity of the antibodies. The authors now cite previous publications in which IHC has been performed for the same proteins, and say that the patterns they observe were compared to these published data (and presumably found to be similar). As far as I can tell (although I may be wrong), the cited articles do not use the same antibodies as the authors have used, so I am not sure of the relevance of these articles. I have no doubt that the antibodies used here localise antigens that are in the appropriate sub-cellular locations – but this hardly proves their specificity. Could the authors please cite articles in which the same antibodies have been used successfully in IHC – thereby supporting their use here. If there is no published use of these antibodies for IHC, I would doubt their specificity unless the authors can demonstrate otherwise (westerns? IHC with positive and negative control tissues known to have high and low expression?).

Points 8/10 related to the interpretation of the miR-21 expression data and its correlation with IHC for the potential miR-21 targets. The authors now present their full dataset, which helps greatly (minor essential revision: some panels are missing the appropriate blue boxes – and the normal, FEA, DCIS and IDC labels are missing on the PDCD4 data). However, I am still concerned that these data are slightly over-interpreted. Specific points:

a) Results (last line): “nuclear PDCD4 staining patterns does show an inverse relation to the miR-21 staining”. This may be just about true for normal and IDC – but is true in fewer than half FEA and only 5 DCIS cases. This needs to be toned down or made more specific for normal and IDC. In any case, the authors conclude it is unlikely to be a direct target.

b) Discussion: “stronger staining [for TM1] in both normal and FEA and... much weaker staining in DCIS and IDC “. Data are only available for 11 cases of DCIS (1 negative; 6 +; 3++ ; 1+++ ) – this is simply not convincingly “much weaker” than the staining in normal (0 negative; 4 +; 10 ++; 0 +++). Please change.

c) Conclusions: “Our results demonstrate that in part of the cases the FEA and DCIS components share a similar miR-21 profile”. This doesn’t really mean
anything as ‘in part’ and ‘similar’ are so vague.

d) Conclusions: “An inversed staining patterns in the normal and IDC components was observed for miR-21 and TM1… supports targeting of TM1 by miR-21”. This statement is true – but ignores the data in FEA and DCIS that do not strongly support this view. Again I think this needs to be toned down.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**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