Author's response to reviews

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

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Author's response to reviews: see over
Dear Dr Scott Edmunds,

We would like to thank you for the opportunity to revise our manuscript entitled: “Expression of miR-21 and protein targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma”.

We have adapted the manuscript according to the suggestions of the second reviewer and uploaded the revised version of our manuscript with changes marked in blue. Our point-by-point response to the reviewers’ remarks is given below. We have added a figure showing our positive control staining results for PDCD4 and TM1 for inspection by the reviewer, because this was the main point of criticism. If necessary we can add this picture also as a supplementary file to our paper.

We thank the reviewer for the comments, which enabled us to improve the quality of the manuscript.

Yours sincerely,

On behalf of all authors,

Anke van den Berg

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Response to comments of reviewer 2

Point 5
We have added an additional reference for immunohistochemistry for PTEN and used the antibody as requested by this reviewer. For PDCD4 and TM1 the papers describing immunohistochemistry used other antibodies other than ours. Some of those are not commercially available. Both antibodies which we used are commercially available, raised against synthetic peptides containing a specific epitope of the protein to be investigated. For TPM1 the peptide sequence is: DRAEQAEADK KAAEDRSKQL EDELVSLQKK LKGTEDELKSEALKDAQE. The first part of this peptide is identical to the peptide used to generate the TM1-antibody clone TM311, which is used in several other publications. We have added two original papers to our reference list. Specificity of the PDCD4 and TM1 antibodies we used has been demonstrated previously by Western blot in a publication by Haraguchi et al., 2009 and by Akgul et al., 2009. Since for the primary antibodies against PDCD4 and TM1 no earlier publications about immunohistochemistry are available, we added a file containing our control staining results for inspection by the reviewer.

We have adapted the text accordingly:
“...The staining patterns for all three antibodies were compared to previously reported staining patterns in these tissues to assess the specificity of the staining [20-22]. For PTEN we used the same antibody as used previously for prostate cancer [23]. For PDCD4 and TM1 specificity of the antibodies was shown previously by Western blot [24,25]. For PDCD4 normal colonic epithelium served as positive and intestinal type colon carcinoma served as negative control tissue. For TPM1 colon served also as control tissue, since the muscularis mucosa and the smooth muscle layer around capillaries should be positive and the colonic epithelium should be negative. Additional negative controls were obtained by omission of the primary antibodies from the staining”.

Points 8-10
We have checked the supplementary tables and added the missing boxes (case 10). Normal, FEA, DCIS and IDC labels have been added to the PDCD4 table. For points a-d we have adapted the text according to the suggestion of this reviewer:

a. However, the nuclear PDCD4 staining pattern does show an inverse relation to the miR-21 staining pattern for normal and IDC. For FEA and DCIS such a relation was not obvious.

b. Our results showed that there was an obvious decrease in the percentage of positive cases for TM1 with progression, i.e. the majority of cases being positive in both normal and FEA and a minority of cases being positive in IDC.

c. Our results demonstrate that in 5 out of 12 cases with simultaneous presentation of normal, FEA and DCIS, the FEA and DCIS components share a similar miR-21 profile.
d. An inversed staining pattern was observed for miR-21 and TM1 in the normal and IDC components, but not in FEA and DCIS. These findings might support targeting of TM1 by miR-21 in breast cancer.