Author's response to reviews

Title: EpCAM Overexpression Is Associated with Downregulation of Wnt Inhibitors and Activation of Wnt Signalling in Human Breast Cancer Cell Lines

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Author's response to reviews: see over
To the

Editorial Office

BMC Cancer

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Re: Manuscript Re-submission

Dear Editor,

please find attached our revised manuscript after consideration of the suggestions of the reviewers. We think that the suggested corrections have improved the quality of the manuscript and we hope that it is now suitable for publication in BMC Cancer. Due to additional experiments that we had to perform a new co-author (Agnieszka Martowicz) was added to the authors’ list.

Please find the revisions on the next page. All the corrections have been underlined in the manuscript.
Revisions:

Reviewer 1:

Major Revisions:

1a: New stably transfected MDA-MB-231 cell clones have been tested and the data of the proliferation assay are very similar to those presented in Figure 4. For this reason we decided not to include another Figure with similar data into the manuscript. If needed, the figures of the new clones might be available as supplementary material. The analyses on different clones is now mentioned in the results section.

1b: The analysis of migration through Matrigel coated PET membranes (BD FluoroBlok™ Assay) has now been shown in Figure 3.

1c: At the moment we have another project dealing with the effect of EpCAM in non-tumorigenic or immortalized breast cancer cells (i.e. MCF10A) or even normal cells. However, these data will be published separately.

1d. We have performed an assay dealing with resistance to chemotherapeutic agent Docetaxel and the data are presented in figure 5. EpCAM positive Hs578T cells appear to be more sensitive to chemotherapy-mediated apoptosis as compared to their negative counterpards.

2. In our view the increase of luciferase activity of 20% in the Wnt signal activity assay is a significant enhancement of signalling and in an expectable dimension, as the MDA-MB-231 cell line expresses endogenous WNT7B (Huguet et al Cancer Res 1994). The association of WNT7B with a more aggressive phenotype has already been described in the literature. In our work, Hs578T cells are used as an example for a low-tumorigenic EpCAM negative cell line, whereas MDA-MB-231 are used as an example for a more tumorigenic one. Therefore we think that the expression of a further oncogene such as EpCAM can enhance cancer associated signalling pathways. However, a substantial basal level of Wnt signalling activity is already present in MDA-MB-231 cells. By choosing adequate negative (containing a non
inducible motif) and positive (constitutive active) controls we could observe an enhanced Wnt signalling activity in MDA-MB-231\textsuperscript{EpCAM} cells in a reproducible manner.

EpCAM does not enhance Wnt signalling in Hs578t cells, the absence of essential partners for Wnt signalling could be a reason for this observation. Expression of Wnt signalling partners is often induced during the process of cancerogenesis. This further supports our statement that EpCAM mediated effects are strongly depending on the “cancerogenic” background of the cells.

The requested western blot with an antibody recognizing the intracellular domain of EpCAM has been performed and has been included in Figure 8.

Minor revisions:

1) The perinuclear localization of EpCAM expression is now mentioned in the result section.

The requested western blot with an antibody recognizing the intracellular domain of EpCAM has been performed and has been included in Figure 8.

2) The labels of all the Figures were corrected and former Figure 7 was eliminated.
Reviewer 2

1) Additional cell clones were tested for proliferation (see comments on reviewer 1) and additionally chemotherapy sensitivity tests were performed and are presented in Figure 5. Enhancement of proliferation is not observable for both cell lines, suggesting that the effect of EpCAM is cell-line dependent. We conclude that the effects mediated by EpCAM expression can differ, depending on the tumorigenic background of the cells. As mentioned above, effect of EpCAM in non-tumorigenic or immortalized cells (i.e. MCF10A) are performed currently, but will be published separately. Additionally, we would like to mention, that the major part of all available breast cancer cell lines are endogeneously expressing the EpCAM protein.

2) The protein level of TCF7L2 in MDA-MB-231 cells are shown in Figure 7.

3) Lamin A/C, a structural component of the nucleus, was used as control protein to confirm the integrity of the nuclear fraction (figure 8).

4) In our view the increase of luciferase activity of 20% in the Wnt signal activity assay is a significant enhancement of signalling and in an expectable dimension, as the MDA-MB-231 cell line expresses endogenous WNT7B (Huguet et al Cancer Res 1994). The association of WNT7B with a more aggressive phenotype has already been described in the literature. In our work, Hs578T cells are used as an example for a low-tumorigenic EpCAM negative cell line, whereas MDA-MB-231 are used as an example for a more tumorigenic one. Therefore we think that the expression of a further oncogene such as EpCAM can enhance cancer associated signalling pathways. However, a substantial basal level of Wnt signalling activity is already present in MDA-MB-231 cells. By choosing adequate negative (containing a non inducible motif) and positive (constitutive active) controls we could observe an enhanced Wnt signalling activity in MDA-MB-231EpCAM cells in a reproducible manner. EpCAM does not enhance Wnt signalling in Hs578t cells. The absence of essential partners for Wnt signalling could be a reason for this observation. Expression of Wnt signalling
partners is often induced during the process of cancerogenesis. This further supports our statement that EpCAM mediated effects are strongly depending on the “cancerogenic” background of the cells.

5) The discussion section was re-written and the statements were corrected. One of the major objectives for publishing our data, that of course seem to be “conflicting” at the first moment, was to demonstrate that the effects of EpCAM expression can not be generalized for one tumor entity but they are highly dependent on the cancerogenic background. This fact should be considered when evaluating EpCAM expression and oncogenicity. We defined this statement more clearly in the discussion.

6) The data of the migration assays have been added and presented in Figure 3. The last sentence was removed.

Minor revisions

1) Figure references were corrected.