Author's response to reviews

Title: Effects of EpCAM Overexpression on 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 second revision of the manuscript after consideration of the suggestions of referee 2. We think that the suggested corrections have further improved the quality of the manuscript and we hope that it is now acceptable for publication in BMC Cancer.

Please find below the revisions with a point-by-point response to the concerns.

With best regards

Gilbert Spizzo
Revisions:

Referee 2:

Major points:

1) The study is in essence a negative study. EpCAM overexpression in the cell lines selected results in a very marginal functional phenotype. This makes it very hard to interpret the data. It is not clear if the authors explored alternative gain-of-function model systems. This major limitation is not addressed by the authors. At a minimum, this limitation needs to be clearly stated by the authors and discussed.

→ We agree with referee 2 that the analyses of additional cell lines as gain-of-function models would be interesting. However, Hs578T and MDA-MD-231 cell lines were the only cell lines from ATCC that were observed to be EpCAM negative or low-expressing. All other well-established cancer cell lines showed EpCAM expression levels similar to stably-transfected cells Hs578T$^{EpCAM}$ and MDA-MD-231$^{EpCAM}$. A possible way to obtain further gain-of-function in vitro models for breast cancer, would be to collect cells from patients with EpCAM-negative breast cancer (i.e. lobular breast cancer) and to cultivate and expand these cells. However, this would be a new project with a long time frame.

The limitations of our study have now been addressed in the “Results” and “Discussion” section. Moreover, the marginal functional phenotype associated with EpCAM overexpression has been explicitly stated in the text.

2) Two breast cancer cell lines are evaluated and the results are not consistent. For instance, EpCAM overexpression appears to increase proliferation in one of two breast cancer cell lines. Similarly, EpCAM overexpression appears to increase Wnt signaling in one of two breast cancer cell lines. This inconsistency makes it difficult to interpret the results. Additional cell lines need to be tested given the discordant results. The authors are correct in
stating that breast cancer is a heterogeneous disease, and the impact of EpCAM may be context-dependent. However, given that only two breast cancer cell lines are analyzed, even this interpretation is speculative. At a minimum, this limitation needs to be clearly stated by the authors and discussed.

The concerns of the referee are correct. Even in breast cancer cells originating from one parental line a heterogenous behaviour has been observed (Jessani et al, Cell Cylce 2005). The discordant results between the two cell lines and the limitations have now been clearly stated in the “Discussion” section.

(3) The authors imply a functional relationship between the increase in Wnt signaling observed following forced expression of EpCAM and increased proliferation. This is not directly investigated experimentally. Of note Wnt signaling is increased in MDA-231 cells, but increased proliferation, and susceptibility to chemotherapy is observed in Hs578t cells, suggesting that there is no such relationship. At a minimum, this discrepancy needs to be clearly stated by the authors and discussed.

The concerns on the correct interpretation of the data are correct. We have now carefully discussed the results accorting to the referee’s suggestions.

(4) The authors make a number of conclusions that are not supported by the data. The text needs to be more precise: In the title the authors state "EpCAM overexpression is associated with downregulation of Wnt inhibitors and activation of Wnt signaling in human breast cancer cell lines." Activation of Wnt signaling was only observed in one of two breast cancer cell lines tested. The title of the manuscript should be revised.

The title of the manuscript has been revised as suggested.
In the final sentence of the Abstract the authors state "Thus, EpCAM overexpression can promote the aggressiveness of breast cancer cells by stimulating cellular mechanisms including Wnt signaling and proliferation, in a cell-dependent manner." The association between EpCAM-mediated Wnt signaling and proliferation was not investigated in the manuscript. The data presented suggest that there is no relationship. This sentence needs to be revised.

→ The final sentence of the Abstract has been revised accordingly.