Author's response to reviews

Title: Initial activation of EpCAM signalling via cell-to-cell contact

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Author's response to reviews: see over
Dear Sabina Alam,

With this letter I wish to reply to the reviewers re-review on our manuscript “Activation of EpCAM signalling via cell-to-cell contact” submitted to BMC Cancer for publication (#1018409528099673).

We fully agree with Dr. William Gillanders on his criticism that substantial controls have been missing in the former version of our manuscript. Along his suggestions, we have conducted additional experiments in order to corroborate the data and now include them in the revised manuscript.

We feel our manuscript has further improved and hope it now meets the high standards for publication in BMC Cancer.

With best regards,

Olivier Gires
In the following, we would like to respond to the reviewer’s comments in a point-to-point manner.

Re-review report from Dr William Gillanders:

Overall the revised manuscript is improved, although not substantially revised. The most significant change is that the discussion has been extensively revised, and the conclusions are now much more in line with the data presented. No significant new additional data is presented, although the data now includes quantitative measures as requested by all reviewers.

Authors: this point is now changed with the inclusion of new data (see below).

Strengths of this manuscript include the fact that the data is clearly presented and, in general, supports their underlying hypothesis that cell-to-cell contact can contribute to EpCAM cleavage. Previously the authors demonstrated that soluble recombinant EpEX (the extracellular domain of EpCAM) can induce EpCAM cleavage. The authors now extend this finding, demonstrating that cell-to-cell contact induces EpCAM cleavage.

Authors: Likewise, we present data to show that nuclear translocation is mandatory for the signalling moiety of EpCAM (EpICD) in order to develop oncogenic effects.

Weaknesses of the manuscript include the fact that the data represent incremental advances over the studies recently reported in Nature Cell Biology, some inconsistencies in the data presented, lack of appropriate controls for the impact of cell density on proliferation, lack of data to support in vivo relevance, and lack of mechanistic insight into regulation of EpICD nuclear localization. Also, although the data is consistent with the hypothesis that EpCAM on the surface of cells is responsible for EpCAM cleavage following cell-to-cell contact, this is not confirmed directly.

Discretionary revisions:

(1) The manuscript would be improved by revisiting Figure 1. Additional controls are necessary as it is not clear that the figure adequately controls for the increased number of cells in the D2 and D3 conditions. Another interpretation of the data is that EpEX is associated with cell number, not cell density. Generally speaking total protein is not typically considered an adequate control for immunoblots. It is not clear if the cells were grown in serum-free media for these experiments (the text of the manuscript seems to indicate that 10% FBS was used). If serum was used, total protein most likely represents protein from the serum. A more informative control would be to compare EpEX to another secreted or cleaved protein present in the supernatant. Alternatively but not necessarily ideal, secreted EpEX could be normalized to total EpCAM present in cell lysates. I agree with the authors that there is clearly more EpEX in the supernatant. However it is less clear if there is more EpEX/cell in the supernatant. The central hypothesis is that cell-to-cell contact induces EpEX cleavage. If this is the case then there should be more EpEX/cell in the supernatant.

Authors: We fully agree with the reviewer. The revised version of the manuscript now includes additional experiments with equal amounts of cells, which were seeded on culture dishes with increasing areas, thereby generating different densities. These novel data are now included in revised Figure 1F and confirm data in Figure 1D and E. More EpEX was shed per cell when increasing cell density.
(2) The manuscript would be improved if the observation that EpICD nuclear localization at the D3 condition is decreased were evaluated in more detail. Currently there are no mechanistic insights into this observation, and it is not consistent with the hypothesis that cell-to-cell contact results in EpCAM signaling. It is more consistent with the hypothesis that cell-to-cell contact results in EpCAM cleavage, and EpCAM signaling under certain conditions. I agree that such an investigation may be outside the scope of this manuscript. The authors may want to revisit the text to make sure this is clear. For instance, the title of the manuscript seems to indicate that cell-to-cell contact results in EpCAM signaling. I think the data in the manuscript suggest otherwise.

Authors: We have changed the text accordingly and put more emphasis on the fact that cell-to-cell contact induces EpCAM cleavage. However, after release of EpICD into the cytoplasm additional mechanisms, which deserve further assessment, regulate EpICD nuclear translocation. This nuclear translocation of EpICD is somewhat dampened in cells grown to confluency.

In this respect, we not only revisited the text, but likewise the assessment of EpICD localization under varying densities. In the former version of our manuscript, the depiction of the quantification of nuclear localization was misleading as it only incorporated a category +/- . We have re-evaluated all samples and now include this refined evaluation in Figure 2B. Unlike suggested by the former depiction, EpICD was present in the cells’ nucleus, but to diminished extent.

(3) The manuscript would be improved if the authors extended the findings to the in vivo setting. I agree that such an investigation may be outside the scope of this manuscript.

Authors: In line with the reviewer’s comment, we did not assess in vivo significance in the present manuscript.

(4) Additional discussion would be helpful to resolve the inconsistency in Figures 2A and 4A. In Figure 2A no EpICD nuclear localization is observed in condition D3, and there is a corresponding decrease in proliferation. In Figure 4A, the c-Myc WB is substantially increased.

Authors: As stated above, the former depiction of EpICD nuclear localization might have been misleading. Actually, EpICD is present in the nucleus even in cells cultured under high density, but to lesser extent. We have changed this to be more explicit and revisited the text accordingly. For the case of MCF7, which are larger cells than HCT-8, we observed the expected decrease of c-Myc induction at day two for cells grown under highest density.