Reviewer's report

Title: Activation of EpCAM signalling via cell-to-cell contact

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Reviewer: Margot Zoller

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The manuscript “Activation of EpCAM signalling via cell-to-cell contact” by S. Denzel et al. describes that EpCAM cleavage and EpICD nuclear translocation and induction of gene transcription requires cell-cell contact. The authors first demonstrate that EpCAM membrane staining decreases with cell density, while the amount of released EpEX increases. Staining for EpEX and EpICD confirms reduced colocalisation with EpICD, which becomes enriched in the cytoplasm and perinuclear/nuclear. Concomitantly c-Myc, CyclinE and A and eFABP transcription becomes upregulated, which is not seen at high density. Notably, transfection of HEK cells confirms that EpICD acts independent of cell density, which was substantiated by HEK transfectants with an EpICD-ER chimeric cDNA. The mechanism which prohibits nuclear localization of EpICD at high cell density is unknown.

Taken together, the authors provide evidence for cell-cell contact dependent EpCAM cleavage and signalling. However, evaluation of proliferative activity, does not provide a convincing functional assay, because high density is known to interfere with proliferative activity. At least an EpCAM knockdown or an EpCAM-negative line should be included for comparison to an EpCAM transfected line. The observation that nuclear translocation of EpICD is hampered at high cell density is interesting, but not evaluated. The authors also should take into account an additional point. In vivo, cells are mostly in direct contact. According to the authors’ presentation this would imply EpCAM downregulation and/or a failure of nuclear import of EpICD? The authors should discuss this aspect.

I recommend considerable shortening of the manuscript as a letter or short communication, a more careful interpretation in view of the in vivo relevance and inclusion of an appropriate control for cell density-dependent proliferative activity.

Minor point
1. FHL2: introduce abbreviation in summary
2. Introduction: 3. sentence incomplete
3. MM, p 5, l 12: stable cell clones
4. Do not repeat the introduction in the result section (e.g. §1, p 8)
5. Fig 1 D, 1 E: include weight markers
6. Omit Figure 2 A
7. Page 8 last § / p 9 §1. belongs into discussion
8. Figure 3: Ratios to actin should be provided. In HCT-8, d1 and d2, and in MCF-7, d2, loading appears to be unequal. I am not convinced about these data

9. Impact of cell density on proliferation should be controlled with an EpCAM-line. It is a common phenomenon that proliferation decreases with increasing cell density

10. Omit Fig. 4 C

11. p 12 heading: omit of

12. Typing mistake p 14, l 13: ectodomain

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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