Reviewer's report

**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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**Reviewer:** William E Gillanders

**Reviewer's report:**

In the manuscript “EpCAM overexpression is associated with downregulation of Wnt inhibitors and activation of Wnt signaling in human breast cancer cell lines,” Gostner et al. explore the relationship between EpCAM expression and Wnt signaling in human breast cancer. In order to study this relationship, they have chosen to use a gain-of-function model system. The main findings of this study include: (1) EpCAM overexpression increases proliferation in one of two breast cancer cell lines; (2) EpCAM overexpression results in changes in the gene expression profile of two human breast cancer cell lines, with downregulation of Wnt signaling inhibitors at the mRNA level; (3) Wnt signaling, as measured by a TCF/LEF reporter assay, is increased in one of two breast cancer cell lines.

The authors have revised the manuscript. In the revised manuscript, the authors successfully address some of the specific concerns of both reviewers. However, a number of major concerns are not meaningfully addressed, and overall the manuscript is not substantially changed. Remaining concerns (major compulsory revisions) include:

(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.

(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.

(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.

(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.

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.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Please note that I am a scientific competitor. We have used RNA interference to study the impact of EpCAM expression in human breast cancer cell lines, including MDA-231. We have observed that specific ablation of EpCAM decreases breast cancer invasion in these studies (PMID: 15313925, 19141643). We have also observed that forced expression of EpCAM has a limited impact on human breast cancer cell lines. We have established a gain-of-function system where we specifically ablate EpCAM and then rescue with constructs that are resistant to RNA interference (PMID: 19524940).