Reviewer's report

Title: A Mouse Model for Triple-negative Breast Cancer Stem Cells (TNBC-CSC) Exhibits an Aggressive Phenotype

Version: 3 Date: 8 October 2011

Reviewer: Jason Herschkowitz

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Major Compulsory Revisions:
1. I do not understand the rationale behind creating an ER, PR, and HER2 triple positive 4T1 cell line. Most researchers believe that human breast cancers divided by these immunohistochemical markers or by gene expression are different entities/diseases and do not just defer in these markers or the pathways they regulate.

2. The methods are poorly written and do not contain any information about the constructs or methods used to overexpress ER, PR, and HER2. Rat HER2 was used. Does this cause an immune response?

3. Likewise, there are no methods describing the antibodies used for FACS (CD24, ALDH1, and CD44). Was an antibody to ALDH1 used or the ALDEFLUOR assay? There are also descriptions in the methods for constructs and shRNAs that I did not see used in the results section.

4. While these are stem cell markers in some tumor types, this remains unproven in 4T1 cells. The tumor growth data and metastasis data is interesting, however limiting dilution transplantation would need to be performed to validate that the CD24+/ALDH1+/CD44high cells do in fact have greater tumor initiating ability than the other cells.

I do not feel that creating triple positive 4T1 cells is relevant to human breast cancer. However, 4T1 cells are a very useful metastasis model with the advantage of being able to be transplanted into immune competent recipients. Identification and characterization of cancer stem cells/tumor initiating cells in this model is warranted.

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

Quality of written English: Needs some language corrections before being published

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