Reviewer's report

**Title:** Gain in cellular organization of inflammatory breast cancer: A 3D in vitro model that mimics the in vivo metastasis

**Version:** 1 **Date:** 30 August 2009

**Reviewer:** Barbara Vanderhyden

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The authors have compared the ultrastructural organization of spheroids formed spontaneously by Mary-X cells with spheroids formed by MCF-7 cells on agarose. There is also limited comparison of the Mary-X spheroids with tumor emboli formed when these cells/spheroids are xenografted. Both spheroids and tumor emboli from Mary-X cells appear to form complex epithelial structures, with numerous microvilli and canalis. Disruption of the spheroids into single cell suspensions leads to nuclear translocation of E-cadherin and loss of plasma membrane integrity, suggesting apoptosis. While the amount of novel information in this manuscript is quite limited, the results are quite interesting and suggest that complexity of cell association may be a component of inflammatory breast cancer.

Reviewer 1 raised a number of concerns related to the origin of the spheroids examined in this study. The Abstract, Introduction and Methods sections refer to Mary-X as a "mouse model of human IBC", and the authors describe the origin of the spheroids as "prepared by mincing the extricated tumor", which suggests that these spheroids are explants. In their response to this Reviewer's comments, the authors have stated that the Mary-X cells are an established cell line that grows as tight, compact spheroids. However, they later indicate that "the spheroids are not passaged like typical 2D monolayers. They are prepared from an extricated IBC/Mary-X tumor." They are also heterogeneous cultures, and "The Mary-X spheroids can be maintained in culture for periods up to three months." These descriptions (isolation from tumors, limited lifespan in culture), make it clear that Mary-X is not a cell line, but the cultures are indeed primary cultures of tissue explants. As such, the comments made by Reviewer 1 regarding these spheroids/explants are reasonable, and some concerns are reiterated here.

**Major revisions:**

1) The authors should revise the descriptions in the Abstract, Introduction and Methods to reflect the accurate identity of Mary-X. This does not take away from the value of this model, but its nature needs to be clearly described.

2) The Methods section also needs to clarify the description of the methods used to generate the spheroids and the tumor emboli. The description of the methods provided in the authors' response to reviewers describes the spheroids as being obtained from minced tumors, but does not clarify the origin of the tumor (i.e. primary or lung metastases) or the method for isolation of the tumor emboli.
3) Since the interpretation/conclusion of the results is largely based on the similarity of the Mary-X spheroids to tumor emboli, additional images that show these similarities in the tumor emboli would be appropriate.

4) As noted by Reviewer 1, the influence of the culture system used to generate the MCF-7 on the type of spheroids obtained needs to be discussed. In their response, the authors have provided the rationale for the use of a specific method for MCF-7 spheroid formation, but they did not provide this rationale in the manuscript. The rationale for not using a culture system that supports more complex organization in MCF-7 spheroids is important. Essentially, it appears that the comparison being made is an IBC/high E-cadherin expressing tumor explant (Mary-X) with a non-IBC/lower E-cadherin expressing cell line (MCF-7), and so it's not surprising that the behavior of these cell lines is different (whether because of the different nature of the cell line or its level of E-cadherin expression). Some indication of the relative level of E-cadherin expression in the Mary-X and MCF-7 spheroids used in this study would also be appropriate.

5) Quantitative assessment of the observations made for the types of junctions present in Mary-X and MCF-7 spheroids should be included. There is no indication of how many spheroids were examined. Some assurance needs to be made that sufficient numbers (ideally with quantitation) have been examined to ensure accuracy of the observations.

**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.