Title: Profiling of normal and malignant breast tissue show CD44high/CD24low phenotype as a predominant stem/progenitor marker when used in combination with Ep-CAM/CD49f markers

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Author's response to reviews: see over
Dear Prof. Ferdinando Mannello

Journal Editorial Office

BioMed Central

Thank you very much for sending us the reviewer's comments which we have found helpful to modify and improve the article. We have responded accordingly point by point to all of the comments made by the 2 referees. We have also integrated the changes in the revised manuscript and we highlighted all added sentences in yellow.

Please find below the the original reviewer comments copied in blue with the reply under each comment in black.

In addition we have copyedited the paper to improve the style of written English and we revised the author's contribution.
Reviewer 1: Minor comments:

Comment 1. At the beginning of the paragraph in the introduction section, the authors implied that CSC are responsible for the death of up to 20% of women of breast cancer due to drug resistance within a short period of time. This is not true.

Response to comment 1: This sentence has been changed to "A subpopulation of tumor cells called cancer stem cells (CSC) are believed to contribute to the failure of breast cancer therapy due to their reported resistance to chemotherapy and radiotherapy".

Comment 2. The last sentence in Introduction “This study set the stage ...............better treatment outcome.” is overstated.

Response to comment 2: This sentence has been changed. The sentence now reads: “We believe this study may provide a better understanding of breast cancer carcinogenesis as well as facilitate the more accurate identification of CSC. Subsequently, these findings might help in monitoring and/or targeting of this population in the future”.

Comment 3. Figure 6 is mentioned in the Discussion?

Response to comment 3: Figure 6 was a typo and thus it was corrected to figure 5. This figure summarizes the data that we have found in the paper.
Reviewer 2: Minor comments:

Comment 1. Please double check all computations for statistical significance -- for example, in Figure 2B the SEM bars are overlapping considerably between CD10+ and [CD44-high/CD24-low]+ groups, and this is apparently significant, and yet the comparison to the [EpCAM+/MUC-1-neg]+ group is indicated as non-significant when the difference appears even greater than for CD10+ (page 14-15). Similarly, it would appear that some comparisons in Figure 3D should be significantly different, but all the relevant comparisons are indicated as non-significant text (page 16). Additional relevant comparisons in Figure 4D also appear like they would be significantly different, but it is not clear if this was tested.

Response to comment 1: We checked all statistical significances and found them to be accurate. For example, in Figure 2B while there were overlapping variations between CD44high and CD10 groups, the differences between these two groups were statistically significant. This is because paired t-test always detected higher number of mammospheres in CD44high/CD24low cells compared to CD10+ cells in every sample tested. On the other hand, the difference was not dramatic enough to indicate significance in other assays (for example, figure 3D) or the difference were not consistent with all samples although the mean value of one of them was more than the other (figure 4D).

Comment 2. Figure 1A - it is odd there are no Ep-CAM-low/CD49f-low cells.

Response to comment 2: The “mesenchymal” fraction” did not show on the figure because it was gated out. A new sentence has been added in the figure legend to clarify this: epithelial cell populations after exclusion of stromal (mesenchymal Ep-CAM\textsuperscript{neg}/CD49\textsuperscript{neg}, hematopoietic CD45+ and endothelial CD31+) cells.
Comment 3. Methods on tissue isolation could be better described. Was processing performed after routine pathological examination? Was any micro- or macro-dissection performed?

Response to comment 3: Yes processing was performed after routine pathological examination. A gross tissue specimen was obtained and macro-dissected by a pathologist. In addition a frozen section of the biopsy was taken and checked with hematoxylin staining to ensure that it contains carcinoma cells. New paragraph was added in the methods and materials to clarify this (page 6).

Comment 4. It is unclear which of the subpopulation of MDA-MB-468 cells were actually tested in the xenograft model. One would predict that subpopulations of CD49f-low MDA-MB-468 cells would be less efficient in tumor formation -- is this true? These results could be better presented in the text, figure panel, or supplement.

Response to comment 4: All MDA-MB-468 are Ep-CAMhigh/CD49f+ as indicated in the supplementary figure 6. This cell line do not have population C or A and therefore all of the cells were selected using ALDH and CD44^{high}/CD24^{low} markers only.

Comment 5. The "additional markers" tested (page 18) could be mentioned in the text itself.

Response to comment 5: All additional markers are now mentioned in page 18.

Discretionary revisions:
Comment 6. The authors ascribe the descriptions "mesenchymal", "luminal", and "myoepithelial" or "basal" lineages to their various cell populations based on literature references. Perhaps it is well established, but it could add to the manuscript if the studied populations (mammospheres or colonies) were
corroborated to indeed exhibit these phenotypes based on other established lineage markers such as CK14, CK18, and SMA.

**Response to comment 6:** All colonies produced in the colony forming assay were either luminal or basal (based on clear morphological appearance) when sorted from Ep-CAM$^{\text{high}}$ or Ep-CAM$^{\text{low}}$ cells respectively. Furthermore, when colonies were trypsinized and checked using flow cytometry, cells were either MUC-1+ (luminal) or CD10+ (Basal).

**Comment 7.** CD10 subpopulations are investigated within "A", and ALDH subpopulations within "B" -- it would be interesting to have performed subpopulation analysis by CD10 in "B" and ALDH in "A" as well for comparison.

**Response to comment 7:** Population A has no ALDH$^{\text{high}}$ cells (page 14) while population B has no significant CD10+ cells (page 15). Therefore this could not be tested.