LETTER

Reply to Maguer-Satta et al.: Revisiting the mammosphere assay

The letter from Maguer-Satta et al. (1) highlights the potential for CD10 to be used as a marker for mammary stem cells based on their work illustrating that FACS-sorted $CD10^+$ cells form bipotent adherent colonies and mammospheres in nonadherent culture (2). In our work (3), we used immunomagnetic beads to isolate luminal and basal/myoepithelial populations through a similar sorting strategy. Although both studies reinforce the notion that epithelial cell adhesion molecule (EpCAM) and CD10 enrich for cells with different functional and phenotypic characteristics (4), Maguer-Satta et al. (1) note a potential difference regarding stem cell activity. In their study, $CD10^+$ cells enriched for mammosphere formation, whereas we reported increased spheres from $EpCAM^+CD10^-$ cells in both adherent and nonadherent conditions. Additionally, consistent with others, we found that bipotent progenitor activity was not restricted solely to basal cells using both in vitro and in vivo assays; hence, we refrained from making conclusions about whether CD10 or EpCAM cells might enrich for stem cells. Rather, we focused on how functional and phenotypic differences in $EpCAM^+$ and $CD10^+$ cells contribute to human breast cancer heterogeneity.

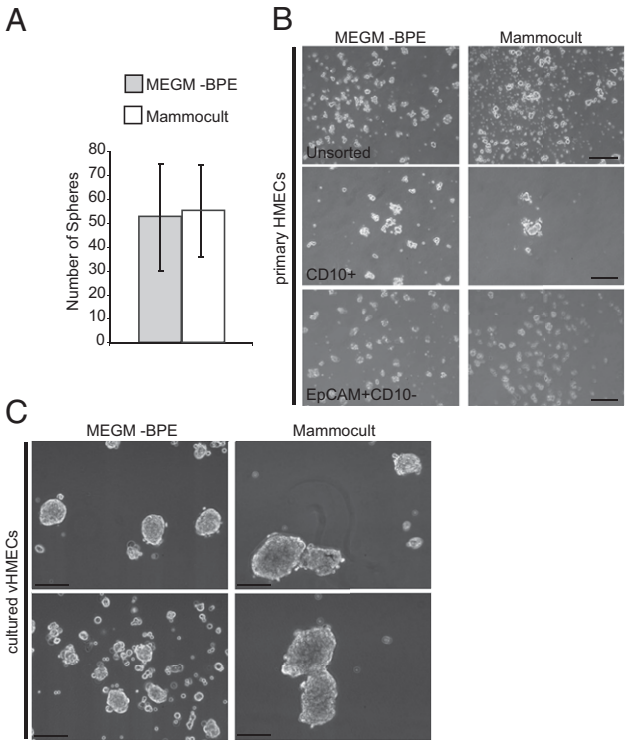

Fig. 1. Media conditions influence sphere size. Primary human mammary epithelial cells (HMECs; *A* and *B*) or cultured HMEC lines derived from unsorted cells [*C*; variant HMECs (vHMECs)] were seeded at 10,000 cells/mL in either mammary epithelial growth medium minus the addition of bovine pituitary extract (MEGM BPE; Lonza) or Mammocult medium in nonadherent culture dishes and allowed to form spheres for 7 d [additional experimental details are provided in our article (3)]. (*A*) Sphere numbers quantified by the Multisizer 3 Coulter Counter. Similar numbers of spheres formed in both culture conditions from unsorted cells. (*B*) Representative images of spheres formed in MEGM-BPE and Mammocult media from unsorted or $CD10^+$ and $EpCAM^+CD10^-$ cells sorted by immunomagnetic beads. (Scale bars: 200 μm.) (*C*) Representative images of spheres formed by vHMECs in MEGM-BPE and Mammocult media. (Scale bars: 200 μm.) Mammocult medium expands sphere size in $CD10^+$ sorted cells and in vHMECs, with the latter showing aggregation of spheres.

We used sphere formation as part of our strategy to demonstrate we had separated two functionally distinct groups of cells for the purposes of addressing how different cells of origin contribute to breast tumor phenotype. As noted, experimental and technical differences likely explain discrepancies between our studies. Although the term "mammosphere" describes spheres generated from primary human mammary epithelial cells in nonadherent conditions (5), we purposefully refrained from using this term in our work because we believe that it is not well-defined in the literature with precise quantitative or qualitative metrics for primary cells (e.g., size, dimension, morphology). Additionally, culture conditions for mammospheres vary, with reports of cells being plated at 20,000 cells/mL (5) to 100,000 cells/mL (2). Because it was reported that plating at 1,000 cells/mL is necessary to ensure that aggregation is not contributing to the formation of mammospheres (5), one cannot exclude aggregation in most reports. In our experience in characterizing cells from nearly 20 patients, spheres vary drastically in number, size, and appearance from patient to patient. Thus, we avoided visualization-based methods to quantify spheres and, instead, used an unbiased metric provided by the Multisizer Coulter Counter. We did note a difference in sphere morphology in the $CD10^+$ fraction, which may reflect the "embryonic body-like" spheres described by Maguer-Satta et al. (1). In addition, we have found that culturing cells in Mammocult medium (Stem Cell Technologies) causes an expansion of the size of spheres formed by both primary cells from the $CD10^+$ fraction and variant human mammary epithelial cells (Fig. 1), suggesting that this medium formulation, designed for enriching the growth of mammospheres, may be selecting for spheres formed by $CD10^+$ cells. Regardless of issues surrounding the mammosphere assay for studying mammary stem cells in vitro, we agree with Maguer-Satta et al. (1) that further investigation and understanding of CD10's role in human breast epithelial cells are needed to understand the behavior of primitive cells within this lineage and their connection to breast tumor biology.

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The authors declare no conflict of interest.

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