Viewpoint

# From normal cell types to malignant phenotypes

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## Abstract

The phenotypic diversity of breast cancer has been proposed to result from different target cell types undergoing oncogenic transformation and giving rise to cancer stem cells. Global gene expression profiling revealed distinct molecular phenotypes and some of these signatures were held to reflect the cell of origin, with the basal carcinomas arising from basal/progenitor cells. Recent work challenges this view by providing evidence that luminal precursor cells are involved in the pathogenesis of basal breast cancers and has made new links between normal cell populations and molecular tumor phenotypes.

With 18 different histopathological entities [1], breast cancer is a particularly complex disease. Global expression profiling of large sets of tumors identified six molecular subtypes; the luminal A and luminal B subtypes (both estrogen receptor alpha-positive), the normal-like, HER2-positive, and basal subtypes (comprising triple-negative cancers) [2], and the claudin-low subtype [3]. Complexity may also exist within a tumor, called tumor heterogeneity [4], where different cell types with varying proliferation capacity, morphology, and protein expression can coexist.

The cancer stem cell theory provides an explanation for tumor heterogeneity. The theory proposes that a subpopulation of tumor cells behaves like normal adult stem cells and the cells self-renew, give rise to different cell types, and account for resistance to therapy [5]. The cell of origin concept – according to which the target cell sustaining the tumorigenic hits becomes a major determinant of the tumor characteristics due to its unique cell context – complements the cancer stem cell theory in explaining tumor phenotypic diversity [6].

Inherent to both concepts is a model of breast epithelial cell hierarchy with a stem/bipotential progenitor at the apex, which gives rise to luminal or myoepithelial/basal progenitors that in turn produce differentiated luminal or myoepithelial cells [7].

Over the past years, evidence has accumulated that supports the existence of cancer stem cells in solid human tumors, including breast cancer [5,8]. Data backing the cell of origin hypothesis, however, have not been forthcoming.

Lim and colleagues have now applied the fluorescence-activated cell sorting approaches – which they and others used so successfully to characterize stem cells and progenitor cells in the mouse mammary gland – in conjunction with different stem cell assays, to the human breast [9]. After depleting dissociated breast cells for endothelial and hematopoietic cells, they distinguished three epithelial cell populations based on differential expression of the surface markers CD49f (integrin alpha 6) and EpCAM (also epithelial-specific antigen). A fourth, double-negative population – dubbed stromal – lacked epithelial markers and expressed PDGFRβ (CD140b).

Among the epithelial cell populations, only the $CD49f^{high}EpCAM^{-}$ population contained cells able to give rise to ducto-lobular structures in the humanized fat pad [8]; this population was named MaSC-enriched, in analogy to the mouse model. In matrigel-based colony-forming assays, cells from the MaSC-enriched population formed heterogeneous colonies, with myoepithelial/basal cells predominating. The $CD49f^{+}EpCAM^{+}$ population was defined as luminal progenitors based on the ability to form luminal colonies in this assay. The third population, $CD49f^{-}EpCAM^{+}$, did not form colonies but expressed luminal genes such as estrogen receptor alpha, progesterone receptor, and cytokeratin 8/18 – and was hence considered mature luminal.

To assess how these distinct populations relate to molecular tumor subtypes, Lim and colleagues took a dual approach. First they used prophylactic mastectomy samples from BRCA1 mutation carriers who develop basal carcinomas at a higher frequency [10] and normal reduction mammoplasties to compare the distribution of the epithelial subpopulations. Strikingly, breast epithelium of BRCA1 mutation carriers was enriched in luminal progenitors and was depleted for the MaSC-enriched population. Furthermore, these luminal progenitors were abnormal and grew independent of an

essential growth supplement; the mature luminal population showed slightly altered expression of progesterone receptor and cytokeratin 5/6.

This elegant genetic approach was complemented by the characterization of the global expression signatures for each cell population, which were compared with the profiles of the six tumor subtypes. The mature luminal signature was associated with the luminal A and luminal B subtypes, whereas the MaSC signature shared properties with the claudin-low and normal-like subtypes. Consistent with luminal progenitors giving rise to basal tumors, the luminal progenitor signature resembled the basal signature.

Just as dust was beginning to settle on the notion that basal tumors originate from stem/bipotential progenitor cells, this work put luminal progenitors in their place. In addition, the research provides a thought-provoking mapping of molecular tumor subtypes to distinct cell populations identified by fluorescence-activated cell sorting.

Yet an alternative to the cell of origin hypothesis cannot be excluded; it is conceivable that the tumorigenic event hits cells at any position in the hierarchy and causes acquisition of luminal progenitor characteristics. This idea is supported for BRCA1-associated tumors by mouse models in which BRCA1 was ablated in different mammary cell types yet the resulting tumors were similar [11,12].

The powerful fluorescence-activated cell sorting approaches hold great promise to further our understanding of human breast biology and carcinogenesis. The major challenges in applying these techniques to highly variable human specimens consist of standardizing every step in the procedure and improving the current stem cell assays with considerable limitations. As an example, a previous report using EpCAM-CD10 sorted populations, mammospheres and induced differentiation assays proposed mammary stem/progenitor cells as the origin of BRCA1-associated tumors, and also reported an increased number of cells positive for the stem cell marker ALDH1 in the breast epithelium of BRCA1 mutation carriers [13] – whereas Lim and colleagues found ALDH1 expression mostly in the stromal population [9].

With further refinements we may soon distinguish more cell types in the human breast and be able to relate changes in cell populations to different hormonal milieus, reproductive stages, genetic changes, and cancer risk.

## Competing interests

The authors declare that they have no competing interests.

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