Breast Cancer RESEARCH

VIEWPOINT

Cell fate takes a slug in *BRCA1*-associated breast cancer

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Abstract

Understanding why *BRCA1* mutation carriers have a predilection for developing clinically aggressive basal-like breast tumors could inform the development of targeted treatment or prevention strategies. Analysis of both mouse and human mammary epithelial cells has identified a role for *BRCA1* in orchestrating differentiation. The ability to isolate discrete epithelial subpopulations from mammary tissue has recently directed attention to luminal progenitor cells – the descendants of mammary stem cells – as the likely 'cells-of-origin' in *BRCA1*-associated breast cancer. A new publication has confirmed the importance of aberrant luminal cells as key culprits and provided insights on how *BRCA1* haploinsufficiency biases luminal cells toward a basal-like fate through aberrant expression of the transcription factor SLUG.

Women harboring mutations in the tumor suppressor gene *BRCA1* (breast cancer 1, early onset) have a profound predisposition to early-onset breast or ovarian cancer or both. *BRCA1*-associated breast tumors are characteristically 'basal-like', containing minimal estrogen receptor (ER), progesterone receptor (PR), and HER2 and expressing 'basal' cytokeratins and epidermal growth factor receptor. Basal-like tumors were originally defined on the basis of microarray studies, in which their molecular signature suggested similarities to basal cells resident in normal breast epithelium. Such observations led to the hypothesis that *BRCA1*-associated tumors arose from stem cells. Moreover, mammary stem cells exhibit a similar 'triple-negative' phenotype.

BRCA1 plays a crucial role in orchestrating the response to double-stranded DNA damage but is recognized to have multiple other functions. *In vitro* cellular assays have indicated roles in regulating mammary epithelial cell proliferation and differentiation and in promoting luminal-to-basal lineage transdifferentiation [1-4]. The ability to fractionate mammary epithelium into different subtypes has enabled insights into target cells prone to tumorigenesis. Using human breast tissue, Liu *et al.* [5] observed that *BRCA1* was required for ER-negative stem/progenitor cells to differentiate into mature ER-positive luminal cells. Lim *et al.* [6] evaluated pathologically normal primary breast tissue samples from haploinsufficient *BRCA1* patients and identified an aberrant luminal progenitor population with factor-independent growth properties. A similar observation was made in *Brca1*-deficient mice [6]. Consistent with a luminal progenitor cell defect, breast tissue from *BRCA1* mutation carriers generally showed an increase in this subset relative to the total epithelial population. Moreover, the molecular signature of luminal progenitor cells was found to be more similar to that of basal-like tumors than to that of any other tumor subtype [6]. Overall, these findings indicated, but did not prove, that luminal progenitors are the 'cells-of-origin' for basal-like tumors arising in *BRCA1* carriers. An important study by Molyneux *et al.* [7] conditionally deleted *Brca1* in different epithelial populations (heterozygous for p53) and revealed that luminal rather than basal cells were predisposed to basal-like mammary tumors. These *in vivo* experiments provided direct evidence that *BRCA1*-associated breast cancers can arise from luminal ER-negative progenitors.

A recent study by Proia *et al.* [8] further highlighted the relevance of luminal cells in haploinsufficient *BRCA1* human breast tissue. With an elegant *in vivo* assay, fresh breast epithelial cells from wild-type or $BRCA1^{+/mut}$ women were simultaneously transduced with potent lentiviruses encoding mutant p53, cyclin D1, activated phosphoinositide 3-kinase (PI3K), and oncogenic K-ras and implanted into humanized mammary fat pads of nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice. Whereas luminal and basal-like tumors arose in mice implanted with wild-type cells, $BRCA1^{+/mut}$ cells largely yielded basal-like tumors,

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indicative of a preprogrammed epithelial defect that dictates tumor phenotype. Proia *et al.* [8] also used epithelial subsets to demonstrate preferential transformation of luminal compared to basal cells.

Gene profiling of wild-type and *BRCA1*$^{+/mut}$ breast epithelia generated a molecular signature enriched for Wnt, Notch, and melanogenesis signaling pathways in *BRCA1*$^{+/mut}$ tissue. Those findings prompted an evaluation of the transcriptional repressor SLUG, which can be activated by these pathways. *SLUG* is a member of the *SNAIL* family and has been shown to have an important role in coordinating the epithelial-mesenchymal transition and programming cells toward a basal-like phenotype in breast cancer [9-11]. *SLUG* is normally expressed in the basal/stem cell-enriched population in both mice and humans [12]. Interestingly, although *SLUG* mRNA levels were unperturbed in *BRCA1*$^{+/mut}$ tissue, abundant levels of SLUG protein were observed. Moreover, knockdown of *BRCA1* by short interfering RNAs in breast cell lines resulted in a twofold increase in SLUG protein. Conversely, knockdown of *SLUG* in breast epithelial cells biased them toward a more luminal cell fate [8]. Thus, BRCA1 appears to regulate SLUG protein stability, and this may directly influence the cell fate specification of luminal progenitor cells. The precise mechanism through which BRCA1 contributes to SLUG protein stabilization remains to be elucidated. A direct interaction between SLUG with BRCA1 was not found, nor did knockdown of the BRCA1-associated RING domain-1 protein (BARD1) alter SLUG levels [8]. The interesting link between BRCA1 and SLUG in perturbing cell fate decisions will undoubtedly form the basis of future studies.

Despite similarities in the epithelial subsets defined by EpCAM and CD49f by Proia *et al.* [8], there are also some noteworthy differences. In contrast to previous authors [6,13,14], Proia *et al.* [8] describe two potentially distinct basal subsets: an EpCAM^{low} population ('basal/myoepithelial') and an EpCAM^{-} population ('mesenchymal' or 'basal progenitor') [15], the latter of which appears to be novel. This subset was found to be expanded in *BRCA1*$^{+/mut}$ breast tissue (with no change in the luminal progenitor subset) and was attributed to diversion of luminal cells toward a basal cell fate. On the other hand, we observed a significant decrease in the basal subset [6] as well as reduced numbers of mammary stem cells in mice. In addition, the mature luminal subpopulation contained a dramatically increased number of CK5/6-expressing cells and fewer PR-positive cells, consistent with a perturbation in differentiation [6]. These differing observations may, in part, reflect different methodological approaches (magnetic beads versus flow cytometry for lineage depletion) and gating strategies used for cell fractionation as well as possible variation between breast samples. Of the 12 *BRCA1*$^{+/mut}$ samples described by Proia *et al.* [8], at least a third of patients had prior breast cancer. Chemotherapy or endocrine therapy may modify epithelial cell composition. Regardless of these differences, both studies identify *BRCA1* as a key regulator of luminal cells.

In summary, one striking consequence of BRCA1 deficiency in mammary epithelium appears to be perturbed cell fate specification, in which luminal cells are biased toward a more basal-like phenotype [8]. Luminal progenitor cells presumably depend, in part, on *BRCA1* for providing high-fidelity DNA repair, as they are highly proliferative. Altered proliferative and differentiative properties, compounded by a predisposition to genomic instability, are likely to set the stage for neoplastic transformation. It seems likely that somatic gene silencing of *BRCA1* through epigenetic mechanisms plays a similarly important role in sporadic basal-like breast cancer. Further elucidation of molecular perturbations resulting from *BRCA1* deficiency will hopefully provide important clues on therapeutic targets relevant to breast cancer treatment and chemoprevention for high-risk women.

Abbreviations

BRCA1, breast cancer 1, early onset; ER, estrogen receptor; PR, progesterone receptor.

Competing interests

The authors declare that they have no competing interests.

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