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A Twist of Cell Fate

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function and heart pathologies. Thus, there is an obvious need to understand the signals and cues that direct cardiomyocyte diversity and promote electrical and mechanical maturation. This goal is challenging using embryos generated with conventional transgenic technologies since many genes have multiple essential roles and their inactivation early on can mask later functions. As a result, PSCs represent a straightforward means to dissect the molecular basis for late-stage developmental or maturational events by bringing in novel technologies such as siRNAs, miRNAs, or small molecules, especially using high-throughput technology.

In summary, embryology redux can lead to efficient, directed differentiation of PSCs, enhancing knowledge of embryogenesis and increasing the relevance of PSC-derived cells for practical applications and research. This and other recent examples of efficient differentiation [e.g., pancreatic endocrine cells and motor neurons (Kroon et al., 2008; Wichterle et al., 2002) bring us one step closer to being able to scrutinize highly complex normal and pathological behaviors of terminally differentiated cells in culture dishes.

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Individuals carrying deleterious BRCA1 mutations typically develop basal-like rather than luminal breast cancers. In this issue of *Cell Stem Cell*, Proia et al. (2011) study breast tissue from women with heterozygous BRCA1 mutations and identify molecular mechanisms that regulate mammary progenitor cell differentiation and bias toward subsequent basal-like tumor formation.

Since the early 1990s, investigators have known that certain families contain women who exhibit a significant increased lifetime risk for breast and ovarian cancer. In this population, the genetic locus responsible for this risk, BRCA1, is heterozygous for deleterious mutations where one copy of the gene is truncated, mutated, deleted, or silenced by DNA hypermethylation (Ostermeyer et al., 1994). Breast tumors that form in these women are unusual on several levels. First, the tumors develop at an early age and are a manifestation of an increased lifetime risk of developing breast cancer. Although several BRCA1 functions may be altered in individuals who bear a mutated copy of the gene, the actual molecular basis for this increased risk is unknown. Second, the tissue types that exhibit an increased likelihood of developing tumors are restricted to a small number, mostly breast and ovarian tissues. Why the increased risk for cancer does not extend to other organs or lineages is also a mystery. Third, and the topic of this Preview, the type of breast cancer that is most typically diagnosed in BRCA1 mutation carriers is of a particular subtype, the highly lethal basal-like subtype. Understanding how the BRCA1 protein participates in any of these three malignant patterns (Venkitaraman, 2002) would be a major step forward in the effort to treat and eventually eradicate the disease. In this issue of *Cell Stem Cell*, Proia and colleagues in the Kuperwasser laboratory make use of primary breast tissue samples isolated from disease-free BRCA1 mutation carriers (Proia et al., 2011). The authors present a carefully crafted study that addresses the origin of the basal-like phenotype that predominates in the tumors that develop in these women. This study constitutes a cornerstone in the field as the use of tissues obtained from women that carry these mutations provides unparalleled opportunities to gain insights into the molecular events that underlie the puzzles noted above. Through a compelling set of supporting data, the authors reveal that the propensity to generate basal-like tumors in breast tissue that

harbors heterozygous BRCA1 mutations derives from a perturbation in breast epithelial cell differentiation caused by a defect in BRCA1 function.

The Kuperwasser laboratory has pioneered the development of sophisticated culture tools that bridge the gap between animal models and human tumor studies (Proia and Kuperwasser, 2006). In the spirit of this approach, they introduced a cocktail of oncogenes into a bulk population of human breast epithelial cells obtained from disease-free women that were either wild-type ($BRCA1^{+/+}$) or heterozygous mutant ($BRCA1^{+/mut}$) and injected the manipulated cells into a murine fat pad that had been cleared of murine epithelial cells and "humanized" by the transplantation of human stromal fibroblasts (Proia and Kuperwasser, 2006). This strategy allows the introduced human epithelial cells to expand in an environment that supports their growth. The authors then monitored the characteristics of the resulting human breast tissues and carefully analyzed the subtype of tumors that formed in this elegant in vivo model. While wild-type cells generated both luminal and basal-like tumors, $BRCA1^{+/mut}$ cells gave rise to an overrepresentation of basal-like tumors. These results provide the first hint that the bias toward forming a basal-like tumor resides in the spectrum of cells present in the breast tissue prior to disease formation. This observation is consistent with a "cell of origin" explanation for tumor subtype since the transforming oncogenes introduced in both wild-type and $BRCA1^{+/mut}$ cells were identical.

In a complementary approach, an in-depth examination of the resident cells present within the patient-derived, disease-free tissue was performed using flow cytometry, mammosphere formation analysis, and in vivo outgrowth assays. The results of these efforts demonstrated that luminal progenitors from $BRCA1^{+/mut}$ tissue exhibit defects in luminal maturation and differentiation and unexpectedly give rise to basal-like differentiation phenotypes. It was further demonstrated that basal-like tumors were derived from luminal progenitors that underwent increased basal differentiation. Strikingly, the authors found that the cell-fate decision that directed progeny toward either a luminal or basal outcome is governed by the activity of the transcription factor SLUG, which falls under the regulation of BRCA1. Thus, haploinsufficiency for BRCA1 protein activity in mutant carriers results in a lack of turnover of SLUG and a subsequent bias toward basal differentiation. To state the converse, SLUG expression regulates human breast progenitor cell differentiation and blocks luminal differentiation. This increased expression of SLUG protein was subsequently observed in $BRCA1^{+/mut}$ tissue prior to disease. Finally, the authors documented that the expression of SLUG protein is both necessary and sufficient for in vitro progenitors to adopt the basal-like phenotype.

As the authors emphasize, their current studies identify one way that BRCA1 may influence tumor subtype but do not preclude other mechanisms. Indeed, the identification of specific factors that contribute to increased breast and ovarian malignancy has been daunting. Given that the BRCA1 gene codes for an extremely large protein with multiple functions and that a wide variety of mutation sites have been documented throughout the protein's different domains, it is possible that some mutations may influence tumor subtype via an alternative mechanism. To date, the BRCA1 protein has been reported to participate in the maintenance of genomic integrity, DNA repair processes, transcriptional regulation, cell-cycle-checkpoint control, and chromatin remodeling through its E3 ubiquitin ligase activity (Boulton, 2006). The exciting finding unveiled in this report is that BRCA1 also participates in cell-fate decisions executed by breast progenitor cells. This conclusion extends the impact of previous published studies that indicated that *BRCA1* might function to regulate mammary epithelial cell morphogenesis and differentiation (Furuta et al., 2005; Liu et al., 2008; Kubista et al., 2002; Hakem et al., 1998). As mentioned above, the finding that BRCA1 mutations promote basal-like tumors in a cell autonomous fashion by increasing the pool of progenitors that exhibit basal differentiation is consistent with a "cell of origin" explanation for tumor subtype. Thus, in an interesting twist of biology, at least in this particular tumor example, the tumor's "mutation of origin" promotes the formation of the specific subpopulation that serves as the "cell of origin" for the basal-like subtype. It will be interesting to determine if other tumor examples also demonstrate such a relationship between genetic alteration and dictation of cell fate.

Identifying the basis of basal-like tumor formation is not simply an academic exercise. The basal-like subtype represents the most lethal and metastatic subtype of breast tumors, and insights into the mechanisms that govern its origins or phenotypes provide new targets to block tumor formation in at-risk populations, or to eradicate existing malignant phenotypes, including metastatic potential (Lord and Ashworth, 2008).

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