IN THE SPOTLIGHT

Tracking Evolution of BRCA1-Associated Breast Cancer

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Summary: Single-cell profiling and computational identification of evolutionary paths to BRCA1-associated tumorigenesis predict that PTEN loss and *TP53* mutation precede loss of wild-type BRCA1 in basal-like and luminal tumors, respectively. *Cancer Discov; 2(6); 486–8. ©2012 AACR.*

Commentary on Martins et al., p. 503 (7).

Women with heterozygous protein-disabling germline mutations in the *BRCA1* gene are strongly predisposed to developing breast or ovarian cancer. BRCA1 is implicated in several cellular processes, most notably in the repair of DNA double-strand breaks (DSB) by homologous recombination (HR; ref. 1). Loss of BRCA1 function may therefore promote tumorigenesis by forcing cells to repair DSBs via error-prone mechanisms such as nonhomologous end-joining, resulting in increased genomic instability and accelerated acquisition of mutations in additional oncogenes and tumor suppressor genes that drive BRCA1-associated tumorigenesis. Examples of such collaborating cancer genes are *TP53* and *PTEN*, which are frequently mutated in BRCA1-associated breast cancers (2, 3).

The vast majority of BRCA1-associated tumors show loss of the wild-type *BRCA1* allele through LOH (4). *BRCA1* was therefore initially viewed as a classical tumor suppressor gene, that is, loss of the wild-type allele by a "second hit" mutation was considered to be the very first tumor-initiating event in *BRCA1* heterozygous cells. This notion was, however, confounded by the fact that normal cells do not tolerate acute loss of BRCA1. Genetic inactivation of BRCA1 in cultured cells induces a rapid proliferation arrest, and homozygous *Brca1*-mutant mice display early embryonic lethality (5). Together, these observations suggested that mutations in other genes should precede loss of the *BRCA1* wild-type allele to render cells permissive to loss of this essential component of the HR machinery. *TP53* was a plausible candidate given its frequent mutation in *BRCA1*-mutated tumors and its central role in the activation of cell-cycle checkpoints following DNA damage. Indeed, homozygous *Brca1*-mutant mice displayed prolonged embryonic survival and in some cases even postnatal viability when crossed onto a *Trp53* knockout background (5). Moreover, analysis of fallopian tube epithelium from *BRCA1*-mutation carriers showed loss of the wild-type *BRCA1* allele in tubal intraepithelial carcinomas (which are the precursors of high-grade serous ovarian cancer), but not in "p53 signature" foci of mutant p53–expressing cells present in the same tissue samples, arguing that *TP53* mutation precedes loss of BRCA1 function in the evolution of *BRCA1*-associated ovarian cancer (6).

In this issue of *Cancer Discovery*, Martins and colleagues (7) provide further evidence for the notion that BRCA1 inactivation may not be the first event in BRCA1-associated tumorigenesis. Using a combination of histologic protein and DNA detection methods (immunohistochemistry, immunofluorescence, FISH), they assessed at the single-cell level the expression status of PTEN and mutant p53 protein, as well as the mutational status of the *BRCA1* wild-type allele in tissue sections from 55 BRCA1-associated breast cancers and 20 sporadic control cases. After counting the number of cells assigned to each of the 8 different states representing all possible combinations of 0, 1, 2, or 3 mutations, they determined the most probable tumor-initiating somatic mutation by identifying (within the 1-mutation class) the state with the largest number of cells (Fig. 1). They applied the same method to the 2-mutation and 3-mutation classes to determine the second and third somatic mutations, respectively. Using this approach, they found 2 main paths of tumor evolution within the BRCA1-associated breast cancer panel. Initial loss of PTEN followed by loss of p53 and/or BRCA1 was observed in the majority of BRCA1-associated tumors with a hormone receptor- and HER2-negative (triple-negative) phenotype. In contrast, PTEN loss was never observed in hormone receptor–positive BRCA1-associated tumors, which showed early loss of p53 followed by loss of BRCA1. Strikingly, many BRCA1-associated tumors contained a substantial fraction of tumor cells that had retained the *BRCA1* wild-type allele. This wild-type allele appeared to be functional, as nuclear BRCA1 foci were observed in tumors with retention of the wild-type *BRCA1* allele, but not in cases with complete BRCA loss.

The findings of Martins and colleagues (7) suggest that loss of the *BRCA1* wild-type allele may not only be a late event, but—at least in a proportion of cases—also a nonessential step in BRCA1-associated breast tumorigenesis, raising the intriguing possibility that in these cases tumorigenesis is promoted by BRCA1 haploinsufficiency rather than by BRCA1 loss. Although no defects have been observed in *Brca1* heterozygous mutant mice, several studies have reported haploinsufficient phenotypes in *BRCA1* heterozygous human cells. Impaired homology-mediated DNA repair and elevated

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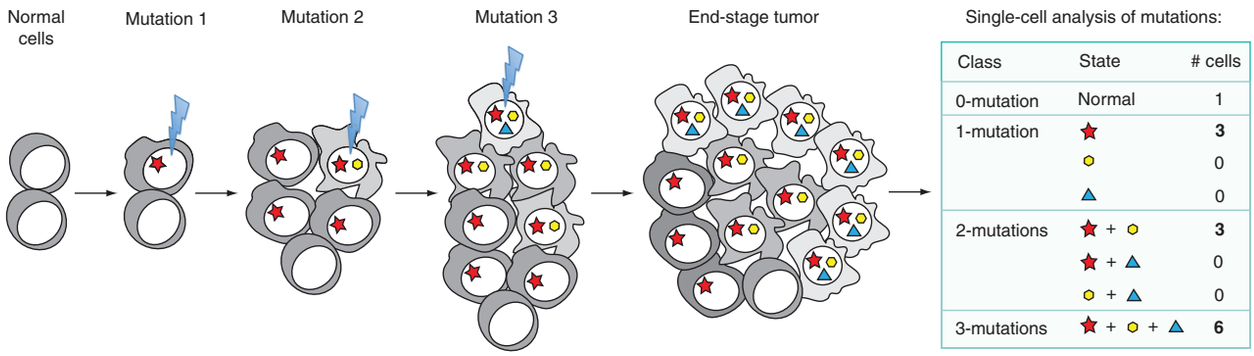

Figure 1. Cancer develops through a multistep process in which normal cells progress to highly malignant tumors via repeated cycles of clonal expansions triggered by (epi)genetic alterations in cancer driver genes. Assuming that invasive tumors still contain cancer cells from earlier steps, one can use single-cell analysis of the driver mutations in the end-stage tumor to track back the evolutionary pathway of cancer development. In case of 3 driver mutations, 8 mutational states can be defined to which each cell can be assigned. Assuming that cells within the 2-mutation class derive from cells within the 1-mutation class, one can identify the most probable tumor-initiating mutation by determining which state within the 1-mutation class contains the largest number of cells. The same approach can be applied to the 2-mutation class to identify the most probable second mutation.

genomic instability was observed in a human cell line engineered to carry a heterozygous *BRCA1*^{185delAG} mutation (8), raising the possibility that *BRCA1* heterozygous cells display a (mild) mutator phenotype that may be aggravated by p53 loss. In line with this, Martins and colleagues (7) found significant numbers of cells with more than 2 centrosomes in *BRCA1* mutation carriers compared with controls. A different haploinsufficient phenotype was reported by several groups, who found that breast epithelial cells from *BRCA1* mutation carriers show defects in progenitor cell lineage commitment (9, 10), resulting in an expanded luminal progenitor population that is thought to be the cell of origin for BRCA1-associated breast cancer (9, 11).

The notion that a substantial fraction of BRCA1-associated breast cancers have retained a functional *BRCA1* wild-type allele may have important clinical implications, as the therapeutic window of novel therapeutics against *BRCA1*-mutated cancer is based on the fact that they specifically target BRCA1-deficient tumor cells but not the normal cells in patients with heterozygous *BRCA1* germline mutations. Indeed, chemical inhibitors of poly(ADP-ribose) polymerase (PARP) display selective cytotoxicity against BRCA1-deficient cells, but they have no selective effect on *Brca1*-heterozygous mouse embryonic stem cells compared with isogenic *Brca1* wild-type cells (12). In line with this, no increase in (the overall very mild) toxicity was observed in *BRCA1* mutation carriers versus noncarriers during phase I trials with clinical PARP inhibitors, such as olaparib (13). Nevertheless, phase II clinical trials showed that PARP inhibitors are very effective against BRCA1-associated breast and ovarian cancers (14, 15).

How can these clinical results be reconciled with the partial loss or complete retention of the *BRCA1* wild-type allele in BRCA1-associated breast cancers, as reported by Martin and colleagues (7)? One possibility is that the heterogeneous responses documented by Tutt and colleagues (14) and Audeh and colleagues (15) may not be due to intertumor heterogeneity or to the fact that the patients in these phase II studies were heavily pretreated with other drugs, but rather result from differences in intratumor heterogeneity with respect to *BRCA1* LOH status. Indeed, intratumor heterogeneity has been recognized as a strong modulator of therapy response and resistance (16). In support of the notion that intratumor heterogeneity in BRCA1-associated cancer may drive acquired resistance, carboplatin-resistant tumor cell clones, marked by secondary *BRCA1* mutations that neutralized the chain-terminating germline mutation, were found to preexist in BRCA1-associated ovarian cancer before carboplatin treatment and tumor relapse (17).

There are alternative explanations for the apparent discrepancy between the clinical efficacy of PARP inhibitors and the incomplete *BRCA1* LOH in BRCA1-associated cancers. PARP inhibition may, for example, display synthetic lethal interactions with (epi)genetic lesions other than BRCA1 inactivation in these tumors. A prime candidate in this respect is *PTEN*, as PARP inhibition was shown to be selectively toxic against PTEN-deficient cells, possibly due to an associated DNA repair defect (18). Because loss of PTEN expression is an initiating event in a large fraction of BRCA1-associated breast cancers (7), it is conceivable that the synthetic lethal interaction with PARP inhibition in these tumors is driven by PTEN loss rather than by BRCA1 deficiency.

A final explanation for the apparent lack of *BRCA1* LOH observed by Martins and colleagues (7) might be that some of the second-hit mutations in *BRCA1* may not be detected by their assays. For example, epigenetic inactivation of the *BRCA1* wild-type allele would not be detectable by FISH. Similarly, certain pathogenic *BRCA1* mutations may still give rise to mutant BRCA1 protein that somehow promotes formation of nuclear RAD51 foci (19). Whether these and similar mechanisms may explain some of the cases with apparent lack of *BRCA1* LOH remains to be elucidated. This undoubtedly daunting task will require the application of additional, more sophisticated methods for single-cell analysis, such as single-cell sequencing (20).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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