IN THE SPOTLIGHT

Tracking Evolution of BRCA1-Associated Breast Cancer
Jos Jonkers

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.

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 results in 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.

The findings of Martins and colleagues (7) suggest that 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 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
genomic instability was observed in a human cell line engineered to carry a heterozygous BRCA1 c.136delC 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.
Grant Support

Work in the author’s laboratory is supported by grants from the Dutch Cancer Society (KWF), the Netherlands Organization for Scientific Research (NWO-ZonMW), the Center for Translational Molecular Medicine (CTMM), and the 7th framework programme (FP7) of the European Union (EuroSyStem and EurocanPlatform Projects).

Received April 25, 2012; accepted April 25, 2012; published online June 8, 2012.

REFERENCES

Tracking Evolution of BRCA1-Associated Breast Cancer

Jos Jonkers