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.

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 in other genes such as TP53 or PTEN, loss of the 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. 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...
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 are derived 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.
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