

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.

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

Author's Affiliation: Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Corresponding Author: Jos Jonkers, Division of Molecular Pathology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Phone: 31-20-5122000; Fax: 31-20-5122050; E-mail: jjonkers@nki.nl

doi: 10.1158/2159-8290.CD-12-0186

©2012 American Association for Cancer Research.

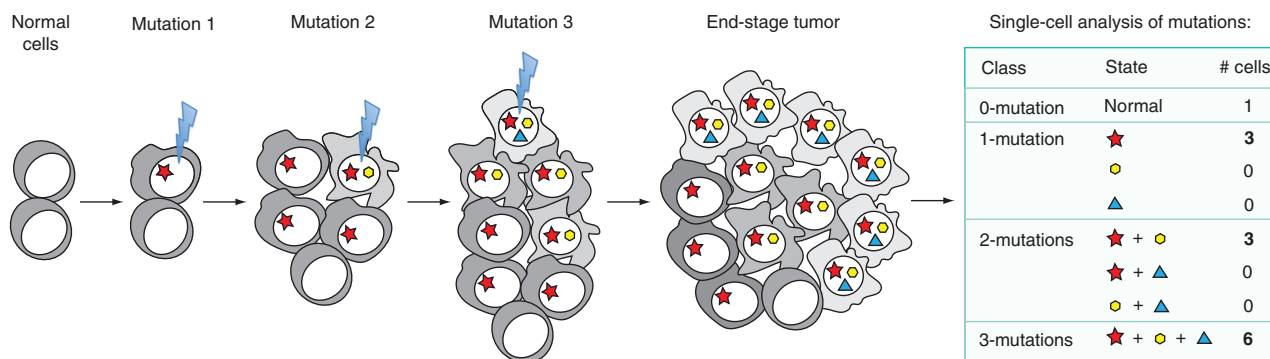


Figure 1. Cancer develops through a multistep process in which normal cells progress to highly malignant tumors via repeated cycles of clonal expansions triggered by (epi)genetic alterations in cancer driver genes. Assuming that invasive tumors still contain cancer cells from earlier steps, one can use 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 *Brc1*-heterozygous mouse embryonic stem cells compared with isogenic *Brc1* 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

- Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. *Mol Cell* 1999;4:511–8.
- Holstege H, Joosse SA, van Oostrom CT, Nederlof PM, de Vries A, Jonkers J. High incidence of protein-truncating TP53 mutations in BRCA1-related breast cancer. *Cancer Res* 2009;69:3625–33.
- Saal LH, Gruvberger-Saal SK, Persson C, Lövgren K, Jumppanen M, Staaf J, et al. Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nat Genet* 2008;40:102–7.
- Cornelis RS, Neuhausen SL, Johansson O, Arason A, Kelsell D, Ponder BA, et al. High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCA1-linked families. *Genes Chromosomes Cancer* 1995;13:203–10.
- Evers B, Jonkers J. Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects. *Oncogene* 2006;25:5885–97.
- Norquist BM, Garcia RL, Allison KH, Jokinen CH, Kernochan LE, Pizzi CC, et al. The molecular pathogenesis of hereditary ovarian carcinoma: alterations in the tubal epithelium of women with BRCA1 and BRCA2 mutations. *Cancer* 2010;116:5261–71.
- Martins FC, De S, Almendro V, Gönen M, Park SY, Blum JL, et al. Evolutionary pathways in BRCA1-associated breast tumors. *Cancer Discov* 2012;2:503–11.
- Konishi H, Mohseni M, Tamaki A, Garay JP, Croessmann S, Karnan S, et al. Mutation of a single allele of the cancer susceptibility gene BRCA1 leads to genomic instability in human breast epithelial cells. *Proc Natl Acad Sci U S A* 2011;108:17773–8.
- Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med* 2009;15:907–13.
- Proia TA, Keller PJ, Gupta PB, Klebba I, Jones AD, Sedic M, et al. Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. *Cell Stem Cell* 2011;8:149–63.
- Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, et al. BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell* 2010;7:403–17.
- Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–21.
- Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–34.
- Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 2010;376:235–44.
- Audeh MW, Carmichael J, Penson RT, Friedlander M, Powell B, Bell-McGuinn KM, et al. Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* 2010;376:245–51.
- Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat Rev Cancer* 2012;12:323–34.
- Norquist B, Wurz KA, Pennil CC, Garcia R, Gross J, Sakai W, et al. Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *J Clin Oncol* 2011;29:3008–15.
- Mendes-Pereira AM, Martin SA, Brough R, McCarthy A, Taylor JR, Kim JS, et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. *EMBO Mol Med* 2009;1:315–22.
- Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, et al. BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. *Cancer Cell* 2011;20:797–809.
- Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, et al. Tumour evolution inferred by single-cell sequencing. *Nature* 2011;472:90–4.

CANCER DISCOVERY

Tracking Evolution of BRCA1-Associated Breast Cancer

Jos Jonkers

Cancer Discovery 2012;2:486-488.

Updated version Access the most recent version of this article at:
<http://cancerdiscovery.aacrjournals.org/content/2/6/486>

Cited articles This article cites 20 articles, 5 of which you can access for free at:
<http://cancerdiscovery.aacrjournals.org/content/2/6/486.full#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerdiscovery.aacrjournals.org/content/2/6/486>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.