

## Commentary

# Role of *BRCA* gene dysfunction in breast and ovarian cancer predisposition

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## Abstract

Tumor suppressor genes that perform apparently generic cellular functions nonetheless cause tissue-specific syndromes in the human population when they are mutated in the germline. The two major hereditary breast/ovarian cancer predisposition genes, *BRCA1* and *BRCA2*, appear to participate in a common pathway that is involved in the control of homologous recombination and in the maintenance of genomic integrity. How might such functions translate into the specific suppression of cancers of the breast and ovarian epithelia? Recent advances in the study of *BRCA1* and *BRCA2*, discussed herein, have provided new opportunities to address this question.

**Keywords:** breast cancer, DNA repair, homologous recombination, ovarian cancer, tumor suppressor genes

## Introduction

Familial breast or ovarian cancer predisposition syndromes have long been recognized. Their genetic bases have become clear with the cloning of two major disease susceptibility genes, *BRCA1* and *BRCA2*, termed herein 'BRCA' genes [1–4]. Each has characteristics of a tumor suppressor gene: inheritance within affected families follows an autosomal-dominant pattern of inheritance; and loss of heterozygosity (LOH) at the relevant gene locus is seen in familial tumors, with retention of the disease-predisposing allele [5–8]. The spectrum of disease-associated mutations includes frequent truncating mutations and less frequent missense mutations. Although LOH is frequently detected at the *BRCA1* or *BRCA2* locus in sporadic breast cancer, the retained allele is almost always wild-type [9,10]. Thus, in contrast to the causal role of

*BRCA* gene mutation in the hereditary syndrome, *BRCA* gene mutation in sporadic breast or ovarian cancer seldom conforms to Knudson's model for tumor suppressor genes [11]. Cancer risk in *BRCA* gene mutation carriers may be increased modestly in other organs. However, highly penetrant, early-onset, site-specific cancer is restricted to the breast and ovary.

*BRCA1* and *BRCA2* homologs have been detected in several mammalian species, including the mouse. *BRCA1* and *BRCA2* are expressed ubiquitously [12–14]. Gene targeting experiments in the mouse [15–22] have revealed functional differences between true null and partial loss-of-function (hypomorphic) mutant alleles of *BRCA* genes. If these distinctions can be made in murine development, the same might be true in human disease. At its simplest,

a hypomorphic *BRCA* allele might exhibit lower penetrance for cancer than a true null allele. Because of the relative scarcity of individual *BRCA* mutant alleles and the existence of unidentified modifier genes, it has been difficult to estimate the penetrance of different disease-predisposing *BRCA* alleles. However, the position of a mutation in the *BRCA1* or *BRCA2* gene can affect the relative incidences of breast and ovarian cancer within a kindred [23,24]. These observations suggest that the breast and ovarian epithelia differ in their requirements for *BRCA* gene function. Whether this is a qualitative or quantitative difference is unknown.

*BRCA1* germline mutations differ from those that affect *BRCA2* with regard to the relative risk of developing other solid tumors. *BRCA2* germline mutation carries an increased risk for cancers of the prostate, pancreas, gallbladder/bile duct, and stomach, as well as for malignant melanoma [25]. *BRCA2* mutation also appears to confer higher risks for male breast cancer [26]. *BRCA1* mutation confers a higher incidence of ovarian cancer than does *BRCA2* mutation [26]. There appear to be histologic differences between *BRCA1*-linked and *BRCA2*-linked breast tumors [27,28]. Taken together, these point to subtle differences between *BRCA1* and *BRCA2* germline mutations in their impact on tumorigenesis. At present, the mechanistic basis of these differences is unknown.

### **Role of *BRCA1* and *BRCA2* in maintenance of genome integrity**

The *BRCA1* and *BRCA2* gene products (*BRCA1* and *BRCA2*, respectively) function in the maintenance of genomic integrity, at least in part by cooperating with recombinational repair proteins. Both *BRCA1* and *BRCA2* form a complex with Rad51, a protein that has an established role in homologous recombination [17,29,30]. The *BRCA1*, *BRCA2*, and Rad51 proteins are coexpressed in the developing mouse embryo, and gene targeting of each revealed similar lethal phenotypes of nullizygous mouse embryos [14,16,17,31,32]. *BRCA1*, *BRCA2*, and Rad51 colocalized in S-phase nuclear foci in somatic cells, and upon the axial element of the developing synaptonemal complex of cells in meiotic prophase I [29,33]. The *BRCA1* and *BRCA2* proteins were also found to be complexed with one another in cell extracts [33], suggesting that these proteins collaborate on a common pathway of tumor suppression. A specific role for this complex in the S phase was implied by the rapid relocalization of *BRCA1*, *BRCA2*, and associated proteins to sites of DNA synthesis after exposure of cells to certain DNA-damaging agents in S phase [34,35]. The *BRCA1* protein also interacts with the Rad50/MRE11/NBS1 complex, implicated in the response to double-stranded DNA breaks [36].

Functional data from the study of *BRCA1*- or *BRCA2*-mutated mice has confirmed a role for these genes in the

maintenance of genomic integrity. Both *BRCA2* and *BRCA1* homozygous mutant cells exhibit ionizing radiation sensitivity, a frequent indicator of a DNA repair defect [17,20]. *BRCA2* or *BRCA1* homozygous mutant mouse embryonic fibroblasts (MEFs) from embryos reveal spontaneous chromosomal anomalies and chromosome breakage, which is consistent with a recombination defect [20,37]. A unique *BRCA1* homozygous mutant embryonic stem cell clone revealed reduced efficiency of gene conversion in response to a site-specific double-stranded DNA break [38]. A defect in gene targeting (a process that is dependent on homologous recombination) in the same embryonic stem cell clone was improved by re-expression of wild-type *BRCA1* [39]. *BRCA1* or *BRCA2* mutated cancer cell lines reveal abnormally delayed kinetics of double-strand break repair (DSBR) [18,40]. Definitive evidence linking *BRCA1* function in DSBR with its tumor suppressor role came from the finding that wild-type, but not clinically defined mutant *BRCA1* alleles, can restore efficient DSBR to a *BRCA1*-mutated breast cancer cell line [41]. The biochemical mechanisms of action of *BRCA1* and *BRCA2* in DSBR have yet to be determined. Where examined, *BRCA* mutant cells revealed defects in homologous recombination, but not in nonhomologous end-joining [22,27,38].

Although recombination would seem to be a unifying theme in these processes, other repair functions, such as the transcription-coupled repair of oxidative DNA damage, are defective in some *BRCA1*-mutated cells [22,42]. *BRCA1* also plays a role in the G2/M checkpoint response to ionizing radiation, although this has not been observed in all *BRCA1*-mutated cells [21,41]. *BRCA1* or *BRCA2* homozygous mutation leads to severe aneuploidy, accompanied by centrosome amplification [21,43].

Genomic instability is characteristic of cancer cells, and it is not difficult to imagine that mutation in *BRCA1* or *BRCA2* might accelerate tumorigenesis, for example through promoting aneuploidy, chromosomal translocation or LOH events. If *BRCA* gene functional inactivation destabilizes the genome, tumor development might be compressed into a shorter time frame. In diseases such as breast or ovarian cancer, the incidence of which increases with advancing age, the 'mere' acceleration of disease progression could contribute to the early onset of disease that is characteristically seen in carriers of *BRCA* mutations. However, this does not provide an obvious explanation of the specifically increased risk of breast/ovarian cancer in *BRCA* gene mutation carriers. The following sections explore hypotheses that could account for this specificity.

### **Collaboration between repair and checkpoint functions in tumorigenesis**

*BRCA1* and *BRCA2* nullizygous embryos die early in development, with a severe growth deficit accompanied

by elevated expression of the p53-responsive cell cycle inhibitor, p21 [16,44,45]. This suggests that not only failed DNA repair, but also the cell's response to that failure might be relevant to *BRCA* gene biology. If *BRCA1* and *BRCA2* are DNA repair genes, then the p53/p21-mediated growth arrest seen in *BRCA* mutant tissue might represent a 'checkpoint' response to spontaneous DNA damage arising as a result of the failure of DNA repair processes. The above-noted chromosome breakage syndrome, described in *BRCA2* or *BRCA1* homozygous mutant MEFs, supports this idea [20,37].

These observations suggest a way to understand the role of *BRCA* gene mutation in tumorigenesis. Perhaps loss of *BRCA* gene function in an otherwise intact epithelial cell might lead to its death or arrest, because of activation of checkpoint functions. However, if *BRCA* gene mutation were to occur within a cell that had already suffered inactivation of critical DNA damage-responsive checkpoints, then the abnormalities in DNA structure resulting from *BRCA* gene loss might be tolerated, and might then manifest their potential as accelerators of tumor progression. This hypothesis would predict that checkpoint loss is a necessary precursor of *BRCA* gene inactivation in tumorigenesis.

Several recent developments in mouse models of *BRCA*-linked disease support this hypothesis. *BRCA1* or *BRCA2* mutant MEFs undergo growth arrest at early passage [20,37]. Both p53-dependent and p53-independent checkpoints appear to play a part in this growth arrest [20,22,46]. Recently, by use of the Cre-lox system, *BRCA1* gene inactivation was achieved specifically in the breast of the adult mouse [47]. Such mice developed late-onset breast cancer, with frequent p53 mutation seen in tumors. When these mice were re-examined on a p53<sup>+/-</sup> genetic background, breast cancer was detected at higher frequency and with earlier onset [47]. This model indicates a permissive role for p53 mutation in *BRCA*-linked disease. Hemizyosity in p53 was also found to play a permissive role for breast tumor development in *BRCA1*<sup>+/-</sup> mice that had been exposed to ionizing radiation [48]. Mutation in p53 is common (occurring in approximately 30%) in sporadic breast/ovarian cancer, but is considerably more common (occurring in approximately 60%) in *BRCA*-linked disease [49]. *BRCA*-linked disease has also been found to be associated with rare p53 mutant alleles, suggesting that novel p53 functions may be lost in *BRCA*-linked tumorigenesis [46,50]. The full spectrum of checkpoint(s) that are responsible for restraining cells mutated for *BRCA1* or *BRCA2* from continued proliferation remains to be defined. One recently identified candidate is the spindle checkpoint [46].

If *BRCA*-linked disease requires inactivation of checkpoint(s) followed by *BRCA* gene loss, then the tissue specificity of *BRCA*-linked disease might arise from a spe-

cific predisposition of the breast and ovarian epithelium to lose the function of such checkpoints. If so, the question shifts sideways – what determines the timing of inactivation of DNA damage checkpoints in breast/ovarian cancers?

### Recombination and breast development

The breast epithelium undergoes distinct developmental programs during puberty and pregnancy. During puberty, in particular, rapid proliferation of breast tissue occurs, and the progeny of this proliferative burst are retained within the breast lobule. This is demonstrated by the finding that lobules of the breast are clonal [51–53]. In this way, breast epithelial cells have the potential to retain 'memory' of genetic alterations that occurred earlier in breast development. In contrast, some other epithelia that are characterized by rapid proliferation, such as the intestinal epithelium, shed cells continuously.

Radiation exposure in young women – in atomic bomb survivors or from iatrogenic causes – carries with it a specifically increased risk of subsequent breast cancer [54,55]. Women who were exposed to A bomb radiation at less than 20 years of age developed breast cancer with normal latency, suggesting that that risk was increased but that disease progression was unaffected. More recently, it became clear that A bomb exposure below the age of 10 years, before the onset of puberty, increased the risk of subsequent adult-onset breast cancer [56]. Perhaps this early impact of radiation exposure on breast cancer risk reflects the 'memory' of breast epithelial stem cells for genotoxic damage. Interestingly, a cohort of women who survived A bomb exposure developed early-onset breast cancer (<35 years of age), suggesting the existence of a susceptible genetic subgroup [57]. It has been suggested that this cohort may represent women with pre-existing *BRCA* gene germline mutations [58].

Do such observations tie in with *BRCA* gene biology? If a *BRCA1*<sup>+/-</sup> or *BRCA2*<sup>+/-</sup> mammary cell were to develop checkpoint defects and undergo LOH at the relevant *BRCA* locus early in breast development, then a number of daughter cells exhibiting this LOH event could be produced and retained within the affected lobule. Cancer risk could be multiplied in the breast epithelium by such an event, in proportion to the number of daughter cells retained after the LOH event (which might, at a maximum, constitute an entire lobule, comprising millions of at-risk epithelial cells). By contrast, other rapidly proliferating epithelia in which daughter cells are rapidly shed (such as the colonic epithelium) would not encounter this risk amplification mechanism. In this way, the clonality of the breast epithelium may, in part, account for the enhanced sensitivity of the breast to genotoxic damage as a mechanism of carcinogenesis.

Furthermore, if the *BRCA*<sup>+/-</sup> genotype exhibits haploinsufficiency, then the risk of such early LOH events might be

increased. This model predicts that the years surrounding puberty are particularly important for *BRCA*-linked disease, and that the breast epithelium may develop detectable genetic lesions in checkpoint and *BRCA* genes before the end of puberty. Although there is relatively little information that is directly pertinent to such mechanisms, one study [59] suggested that LOH events connected with *BRCA*-linked disease might occur early in breast development.

### Mutagenesis models

At first glance, generic cellular functions such as DNA repair/recombination would seem unlikely candidates for having tissue-specific functions. However, the precise genetic consequences of *BRCA* gene inactivation have yet to be fully defined. Defects in DNA repair are frequently accompanied by an increase in the mutation rate. An interesting example of potentially tissue-specific effects of mutagenesis came from a study of the impact of mismatch repair (MMR) defects on colon cancer. MMR dysfunction gives rise to characteristic frame-shift mutations across certain nucleotide repeat sequences. In MMR-defective colon cancers, frame-shift mutations were detected repeatedly within a purine-rich sequence in the type II transforming growth factor- $\beta$  receptor gene sequence, resulting in its inactivation [60]. This suggested that MMR defects might promote colon cancer specifically by virtue of their characteristic mutagenic 'signature'. Could an analogous effect connect *BRCA* gene dysfunction to breast/ovarian cancer? There is as yet no indication that *BRCA* gene inactivation gives rise to a mutagenic event that is capable of delivering such specificity. However, 'forward mutagenesis' studies, which can provide unbiased information regarding mutagenesis, have not yet been reported for the *BRCA* genes. The full spectrum of mutagenesis attributable to *BRCA* gene inactivation is therefore unknown.

One similarity between the breast and the ovary is their regulation by estrogenic hormones. A positive correlation has been observed between estrogen exposure and breast cancer risk [61]. This effect may in large part reflect proliferative effects of estrogen upon its target tissues. In addition, however, some estrogen metabolites, which might be expected to accumulate in estrogen target tissues, have been shown to chemically modify DNA *in vitro*, and can promote carcinogenesis in some rodent models [62,63]. An estrogen target tissue might therefore suffer increased DNA damage directly from estrogen metabolites, giving rise to a 'remote carcinogenesis' mechanism (i.e. although potentially carcinogenic in other tissues, the pharmacokinetics of the carcinogen dictates a restricted site of action *in vivo*).

A defect in recombination could amplify the carcinogenic potential of this tissue-specific DNA damage. For example, work in prokaryotes has revealed a key role for homo-

logous recombination in maintaining genomic integrity after DNA polymerase stalling/replication arrest (for review [64]). Bulky DNA adducts, such as those formed by estrogen metabolites, might be expected to induce DNA polymerase stalling when encountered by the replication machinery, placing particular stress on efficient recombination to prevent genomic instability. Error-prone recombination can give rise to chromosome translocation, LOH events, and other large-scale genome rearrangements that are characteristic of tumor cells (for review [65]). One can imagine how tissue-specific DNA damage, *BRCA* gene dysfunction, and the clonal expansion of breast epithelial cells within the lobule (as discussed above) might collaborate to promote breast cancer above other cancers in *BRCA*-linked disease.

Clearly, the interplay between genotoxic damage and carcinogenesis is not limited to the breast. The gastrointestinal tract, for example, must handle heavy loads of genotoxic agents. However, this epithelium may be protected by the rapid shedding of epithelial cells, which would ensure that only stem cells could potentially form tumors. Such a difference in the physiology of these epithelia may make the gastrointestinal tract less prone to a recombination defect than the breast. At the same time, experimental evidence for this concept is lacking.

The accumulation of a carcinogen at the target site would seem to be a prerequisite for local carcinogen action. This process might therefore also involve other genotoxic agents that accumulate in the breast epithelium or surrounding fat. Hints of this are seen in the property of human mammary lipid extracts to promote single-stranded DNA breaks in cultured primary human mammary epithelial cells [66].

### Transcriptional functions of *BRCA1* and *BRCA2*

*BRCA1* and *BRCA2* have each been proposed to function as transcriptional regulators [67–70]. Indeed, *BRCA1* and *BRCA2* can form complexes with various transcription factors and chromatin remodeling proteins [71–75]. If the *BRCA* genes regulate the expression of a specific set of target genes, then the identification of these targets might reveal tissue-specific functions of *BRCA1* or *BRCA2* that are relevant to breast and ovarian cancer. Several candidate target genes of *BRCA1* have been identified. Notably, some of these are DNA damage/stress responsive genes and, in some cases, are *p53* dependent [76–78]. Both *BRCA1* and *BRCA2* can interact with *p53* [79–81]. Overexpressed *BRCA1* is toxic to cells and can stabilize *p53* [82,83]. In view of the repair functions of the *BRCA* genes and the genetic interactions between *p53* mutation and *BRCA* gene mutation, discussed above, the relationship between *BRCA* gene products and *p53* may be complex. Evaluation of *BRCA*-*p53* interactions may reveal novel functions of *p53* [46,50].



Transient overexpression of *BRCA1* can modify estrogen receptor-dependent promoter functions [84]. However, estrogen receptor mutation is a frequent event in *BRCA*-linked breast cancer, suggesting that tumorigenesis caused by *BRCA* gene mutation affects pathways other than those controlled by the estrogen receptor. A broader analysis of the physiologic effects of *BRCA* gene products on transcription functions may clarify which genes are directly transcriptionally regulated by *BRCA* gene products, and which of these are relevant to tumor suppression.

## Conclusion

It is not yet clear which properties of the *BRCA* genes account for their tissue-specific actions. Genome stability and transcription functions may each be relevant to *BRCA* gene-mediated tumor suppression. How such functions are applied to the breast and ovary may become clear from a more detailed understanding of the biology of the *BRCA* genes and of these epithelia.

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