Breast cancer risk in BRCA1 mutation carriers: insight from mouse models

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Since its identification 20 years ago, the biological basis for the high breast cancer risk in women who have germline BRCA1 mutations has been an area of intense study for three reasons. First, BRCA1 was the first gene shown to associate with breast cancer risk, and therefore serves as model for understanding genetic susceptibility. Second, the type of breast cancer that occurs in these women has specific features that have engendered new hypotheses about the cancer biology. Third, it is hoped that understanding the origins of this disease may provide the means to prevent disease. Resolving this question has proven extremely challenging because the biology controlled by BRCA1 is complex. Our working model is that the high frequency of basal-like breast cancer in BRCA1 mutation carriers is the result of a self-perpetuating triad of cellular phenotypes consisting of: (i) intrinsic defects in DNA repair and centrosome regulation that lead to genomic instability and increases spontaneous transformation; (ii) aberrant lineage commitment; and (iii) increased proliferation due to in large part to increased IGF-1 activity. We propose that the last is key and is a potential entree for preventing breast cancer in BRCA1 mutation carriers.

Key words: BRCA1, breast cancer, genomic instability, mammary gland, mouse models, stem cells

the biological consequences of BRCA1 germline mutations
The observation that Ashkenazi Jewish women preferentially develop aggressive cancers early in life, led to identification of BRCA1 gene and to new understanding of the biology of breast cancer (reviewed herein and in [1]). Women carriers have up to 80% risk of developing breast cancer, as well as a 40% risk of ovarian cancer. Moreover, cancers associated with BRCA1 mutations are frequently negative for estrogen receptor, progesterone receptor, and HER2 amplification, so-called triple-negative breast cancers (TNBC), which is usually associated with a very poor prognosis.

Prophylactic mastectomy specimens from BRCA1 mutation carriers also have a high frequency of premalignant histopathological lesions not detected by physical examination or radiological assessment [2]. Gross cystic disease is also associated with increased risk of breast cancer [3].

experimental models and phenotypes of Brca1 mutation
The mammary glands of mice with Brca1 deficiency exhibit different degrees of abnormal morphogenesis characterized by blunted ductal morphogenesis (or in some models dilated mammary ducts), hyperplastic alveolar nodules, and neoplasia reviewed in [4]. Mice with homozygous mutations in the Brca1 gene do not survive. To circumvent the problem, the Deng laboratory excised exon 11, so-called Δ11, from the gene in the mammary gland by MMTV-Cre [5]. Both Kim and Xu noted the late development of carcinoma, but only in a small number of animals. For that reason, the Deng laboratory bred Brca1 deficient animals with p53 deficient animals to produce enough animals to study effects on cancer development that occurs between 7 and 17 months of age.

Brca1 deficiency and genomic instability
Genomic instability, aneuploidy, and centrosome aberrations are frequent early events in epithelial cancer [6–8]. BRCA1 functions to control DNA damage response [9] and centrosome replication [10]. Constitutive activation of downstream signaling pathways that endow epithelial cells with an increased rate of proliferation also lead to spontaneous DNA damage and genomic instability that promote a premalignant state [11, 12]. Breast cancers of BRCA1 mutations carriers have more chromosomal abnormalities than sporadic breast cancers. Similarly, mice deficient in full-length Brca1 have decreased expression of genes that are involved in the spindle checkpoint and have an impaired DNA damage response (reviewed in [13]). Genomically unstable cells are most susceptible to malignant transformation [14].

Approximately two-thirds of invasive breast cancers are aneuploid. Numerical and structural centrosome abnormalities are hallmarks of almost all solid tumors and centrosome deregulation has been implicated in the rapid generation of multipolar mitoses and chromosomal instability. Centrosome size and centrosome number show a positive, linear correlation...
with aneuploidy and chromosomal instability in breast cancer [7]. Centrosomes are small cellular organelles, which organize the mitotic spindle during cell division but are also involved in cell shape and polarity. Accurate control of centrosome duplication is critical for symmetric mitotic spindle formation and thereby contributes to the maintenance of genome integrity. Centrosome aberrations can arise as a consequence of abortive mitotic events that elicit a defective centrosome checkpoint or centrosome aberrations can arise in normal, diploid cells and precede genomic instability. Ionizing radiation induces aberrant centrosomes, which may contribute to its carcinogenic action, in part by affecting BRCA1 expression [15].

Moreover, the inherent asymmetry of centrosomes in which duplication of mother centriole generates the daughter centriole endows the two centrioles with different characteristics after cell division. This inherent asymmetry of centrioles has been shown to regulate the orientation of the mitotic spindle in which the cell that retains the mother centriole is retained in the stem cell niche whereas the other cell that receives the daughter centriole moves away from the niche to differentiate [16]. As a consequence, centrosome aberrations can promote tumorigenesis by not only increasing aneuploidy but also because spindle misalignment can compromise cell-fate-determinant function during stem cell division and increasing number of stem or progenitor cells.

**Brca1 deficiency and mammary lineage**

Dysregulated epithelial stem cell number itself is speculated to promote tumorigenesis in a particular tissue [17, 18]. Lifetime breast cancer risk correlates with factors that promote stem cell proliferation [19]. In mouse models, genetic manipulation of β-catenin transcriptional activity increases stem cells, reduces tumor latency, and increases tumor display of progenitor cell markers [20, 21], while increased symmetric division in the Tpr53 null mammary epithelium is thought to contribute to the high rate of transformation [22]. One idea that underlies the interest in stem cells is that DNA damage in stem cells can be ‘fixed’ by passing it on to daughter cells that then expand further, each round of subsequent replication being the occasion for more mistakes. This is countered by the primordial strand hypothesis in which stem cells pass on the replicated strand of DNA to daughters, retaining the unreplicated template in pristine condition [23–25]. However, because stem cells must not only be capable of producing many differentiated progeny, but able to switch between self-renewal and differentiation when appropriate, stem cell self-renewal can also be affected during tissue regeneration after injury from carcinogen exposure [26]. Hence, an early increase in stem cell number may itself be a factor in cancer development [27].

An example to illustrate the potential consequences of stem cell deregulation comes from our studies of radiation carcinogenesis [28]. We used a novel mammary chimera model that consists of clearing the mammary gland of endogenous parenchyma at 3 weeks of age, irradiating the mouse at 10 weeks, and transplanting the cleared mammary fat pads 3 days later with syngeneic Tpr53 null mammary fragments. The doses (10–100 cGy) used in this study are epidemiologically associated with increased breast cancer in women, and could be delivered as a result of repeated CT scans or as a result of radiotherapy (depending on the site and mode of therapy). Breast carcinomas from unirradiated Tpr53 null transplanted to hosts previously irradiated occurred sooner and grew faster compared with controls. Surprisingly, the predominant type of tumor shifted from estrogen receptor (ER) positive to negative. Consistent with the cell of origin hypothesis that ER-negative tumors arise from stem or progenitor cells, expression profiling of tumors from irradiated hosts also showed a strong signature of mammary stem cells. Notably, a similar enrichment for the mammary stem cell signature was found shortly after irradiation of intact mammary gland. We hypothesized that the increase in ER-negative tumors in irradiated mice was due to a radiation-induced expansion of mammary stem cells, which was supported by functional assays of mammary stem cells and activation of the Notch signaling pathway. Remarkably, this mouse model recapitulates the biology of breast cancer following radiation therapy for childhood cancers, in which ER-negative tumors are significantly enriched [29].

A unique feature of the BRCA1 deficient breast is aberrant lineage commitment. Several laboratories using different approaches, including marker analysis, cell surface antigens, and functional assays have documented altered epithelial lineage commitment in both human tissue and mouse models [30–35]. The importance of this in breast cancer stems from the cell of origin hypothesis, which was recently comprehensively reviewed by Visvader [36]. This hypothesis poses that the heterogeneity of breast cancer is determined by the cell of origin’s position within the normal breast epithelial hierarchy and that upon transformation its fundamental programming remains evident in the biology, behavior, and signature of the cancer subtype [36]. This hypothesis suggests that stem cells give rise to tumors that are less differentiated, ER-negative and more aggressive, such as the claudin-low and basal-like cancers, and that more differentiated cells give rise to the ER-positive luminal types [18, 37].

Basal-like breast cancers arising in women carrying mutations in the BRCA1 gene are thought to develop from mammary stem or progenitor cells [18, 37], but there has been considerable debate as to which specific cell in the hierarchy is the culprit. Knockdown of BRCA1 in primary breast epithelial cells leads to an increase in cells displaying ALDH1, a stem cell marker, and a decrease in cells expressing luminal epithelial markers and ER [34]. In breast tissues, loss of heterozygosity for BRCA1 was seen in ALDH1-positive lobules but not in adjacent ALDH1-negative lobules, which suggests that the loss of BRCA1 function blocks epithelial lineage commitment of ER-positive cells.

A study by Lindeman and colleagues [33, 38] identified a luminal progenitor cell using surface markers and provided evidence that suggests that these ER-negative cells are expanded in BRCA1 mutation carriers. Cell surface marker analysis showed that tissue heterozygous for a BRCA1 mutation (BRCA1-mutant samples) contained substantially fewer cells in the mammary stem cell enriched (basal) subset (CD49fhiEpCAM−) but an increase in the luminal progenitor
interplay between BRCA1 and IGF-1

Mammary development is mediated by the ovarian-pituitary hormonal axis. Rodents have been used to define the critical endocrine signals in mammary development (see [39]). While estrogen (E2) alone stimulates mammary ductal morphogenesis, the combination of estrogen together with progesterone causes lobulo-alveolar development similar to that seen during pregnancy. The pituitary gland is essential for mammary development, as demonstrated by the absence of mammary development in hypophysectomized animals, even if E2 is administered in high concentration. Pituitary derived growth hormone (GH), together with E2, is responsible for ductal morphogenesis, while estrogen, progesterone, GH, and prolactin interact to stimulate lobulo-alveolar development in preparation for lactation. Studies in mice suggest that all known actions of GH in mammary development are mediated by IGF-I. IGF-I has been shown to substitute for GH in mammary development in hypophysectomized animals [40, 41] and neither E2 nor GH has any effect on ductal morphogenesis unless animals are also treated with IGF-I. Indeed IGF-I alone was capable of stimulating some degree of ductal branching in the complete absence of GH, E2, and progesterone [41]. Increasing IGF-1 action in normal animals increases proliferation in mammary glands together with greater phosphorylation of target proteins, IGF-IR, ERK, and AKT.

As it is a necessary signal for epithelial proliferation, it is not surprising that IGF-I also plays an important role in the proliferation of early lesions and neoplastic malignancies [39]. We recently tested the idea that inhibiting IGF-1 would be effective in reducing aberrant proliferation, as shown for tamoxifen. Pasireotide is a somatostatin analog that blocks IGF-I action within the mammary gland and also lowers serum IGF-I by inhibiting secretion of GH [42]. Female rats that are both hypophysectomized and oophorectomized at 21 days develop hyperplasia when treated for 7 days with high doses of human GH and follicular phase levels of E2 [43]. Pasireotide was as effective as tamoxifen in inhibiting proliferation and hyperplasia.

Mutation or deficiency of BRCA1 leads to exaggerated estrogen, progesterone, and IGF-1 activity with a major effect on increased cell proliferation. The BRCA1 gene inhibits ER signaling in transfected cells [44], ER activity [45], and estrogen inducible gene expression [46]. It also regulates progesterone receptor signaling activity, and inhibits IGF-1 action [47, 48]. All of these are direct effects with E2 binding to ER and acting as a co-repressor and BRCA1 binding to the IGF-IR promoter and suppressing transcription of IGF-IR mRNA. Breast tumors from BRCA1 mutation carriers have elevated IGF-IR and IGF-I levels [49, 50]. BRCA1 also has important independent actions on DNA damage response [51], stem and progenitor cell fate [34], cell cycle regulation [52] and telomerase activity [53] among others. Deficiency of the mRNA or protein disrupts these functions.

BRCA1 deficiency also leads to increased expression of several insulin-like growth factor 1 (IGF-1) signaling axis members in multiple experimental systems, including mice, primary mammary tumors, and cultured human cells [47, 48]. It also increases IGF and progesterone activity [44, 46]. As IGF-I is permissive for E2- and progesterone-induced proliferation, blockade of IGF-I activity should, and does, inhibit its own action and proliferation induced by E2 and progesterone.

Moreover, experimental models show that increasing IGF-1 activity or expressing a constitutively active receptor can itself independently promote breast cancer [54]. Understanding that fundamental IGF-1 biology drives proliferation in the mammary gland led to a trial in which a somatostatin analog, octreotide, was used together with tamoxifen for breast cancer prevention [55]. However, no benefit was evident. Why? Not all somatostatin analogs have the same action. Octreotide is a strong somatostatin receptor ligand whose major action is reduction in circulating GH largely through SSTR2. Pasireotide also lowers GH but it also has a strong tissue-specific IGF-1 inhibitory action in the mammary gland. Based on our animal data and the similar action of a IGF-1 small molecule inhibitor, we believe that the primary action of pasireotide in the mammary gland is IGF-1 inhibition [42].

chemoprevention in BRCA1 mutation carriers

In women who have sporadic breast cancer, tamoxifen, and the related raloxifene, target E2-mediated proliferation. There is clear evidence of efficacy of prolonged treatment in certain populations, but each has limitations. Raloxifene may not be applicable to premenopausal women, and tamoxifen has low acceptance rates [56]. More importantly, these therapies only target proliferation in response to ovarian hormone action [43], yet breast cancer is clearly not just a disease of proliferation. Genomic instability and aberrant lineage commitment in BRCA1 mutation carriers are critical to the progression to invasive cancer. As noted above, several studies have shown that BRCA1 loss or mutation affects mammary lineage, which in turn affects the rate and type of cancer that develops [18, 32-34, 38]. The experimentally demonstrable aberrant lineage commitment or stem cell deregulation is thought to explain the particular risk of triple-negative breast cancer. A feedforward loop of increased activity of IGF-1 is a distinct contributor to the heightened responsiveness to ovarian hormones, but BRCA1 mutations can also autonomously increase proliferation. Together, the concurrent phenotypes stemming from BRCA1 mutations interact in a more complex pathogenesis than the sequential acquisition of genetic and epigenetic events in sporadic cancer.

The increasingly more detailed documentation of these phenotypes in both human tissue and novel mouse models give credence to the contention that breast cancer is preventable in this population. An ideal therapy would remove the germline mutations but this is unlikely in the foreseeable future. BRCA1
deficiency reduces DNA repair, increases genomic instability, increases proliferation and skews the mammary lineage; thus targeting any one, while leaving the others, could delay cancer, which would certainly be a start. Finding a pharmacological target requires new approaches. A novel proposal is to use PARP inhibitors to selectively eliminate BRCA1 mutant cells that have undergone loss of heterozygosity, based on the paradigm of synthetic lethality [57]. The growing knowledge of BRCA1 biology has given rise to several provocative questions. If the basis for cancer risk is due to defective DNA repair, as is widely believed, why are not all tissues at risk? If proliferation is necessary, why are not antiestrogens effective in this setting? And if the cell of origin is the defining factor for the increase in triple-negative breast cancer, why is there such conflicting evidence experimentally? Eradicating the risk of breast cancer in this population will require defining and removing the lynchpin encoded by BRCA1 deficiency that promotes malignancy in this specific tissue.

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references


