The Complex Relationship between BRCA1 and ERα in Hereditary Breast Cancer
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Abstract  Breast cancer 1 (BRCA1) was initially identified as one of the genes conferring genetic predisposition to both breast and ovarian cancer. One of the interesting aspects of BRCA1-linked cancers is the observed specificity for estrogen-responsive tissues such as breast and ovary. Recent advances in our understanding of BRCA1-linked breast cancers have revealed a complex relationship between BRCA1 and estrogen receptor α (ERα) signaling. Estrogen stimulation increases expression of BRCA1 at the mRNA and protein level and conversely BRCA1 functions to both induce ERα mRNA expression and act as a negative regulator of ERα signaling. Here, we review the relationship between BRCA1 and ERα and discuss the use of antiestrogen therapies such as tamoxifen and aromatase inhibitors in the treatment of BRCA1 mutation carriers.

Background

Introduction. Approximately 3% to 5% of breast cancers arise as a consequence of highly penetrant mutations in the BRCA1 tumor suppressor gene (1). BRCA1 mutation carriers have a 50% to 80% risk of developing breast cancer and a 16% to 40% risk of developing ovarian cancer by age 70 years (1–3). In addition, carriers are at an increased risk of developing uterine and cervical cancers (4, 5). To date, ~300 mutations within the BRCA1 gene have been identified including small insertions, deletions, and nonsense mutations, most of which lead to a functionally inactive protein (6–8). A number of studies have also shown epigenetic inactivation of BRCA1 in sporadic breast cancer, suggesting it may play a greater role than previously suggested (9–12).

BRCA1 has been implicated in a number of important cellular functions including DNA damage repair, cell cycle checkpoint control, apoptosis, and transcriptional regulation (13). The only known enzymatic activity linked to BRCA1 is its ability to function as an E3 ligase in association with its binding partner, BARD1, and it has therefore been suggested that this E3 ligase activity may underpin many of the functions ascribed to BRCA1 (14).

One of the most interesting aspects of BRCA1 biology is the apparent specificity for hormonally regulated tissues such as breast and ovary, despite performing an apparently fundamental role in all cell types. This has led to speculation as to the potential relationship between BRCA1 and hormonal signaling in breast cancer. Paradoxically, ~90% of BRCA1-linked tumors are estrogen receptor α (ERα) negative and, similar to ERα-deficient, tumors have a poor prognosis (15). ERα negativity has also been reported to be a positive predictor of BRCA1 mutation status as many of the characteristics of ERα-negative tumors are also evident for BRCA1 mutant tumors (16, 17). Furthermore, ERα negativity is associated with reduced BRCA1 expression and there seems to be a correlation between the expression levels of BRCA1 and ERα mRNA levels in sporadic breast cancers (18–20). Further information on the relationship between BRCA1-linked tumors and the various subtypes of breast cancer has been elucidated from microarray studies. Microarray-based expression profiling has shown that breast tumors can be classified into at least five major subtypes including ERα-positive luminal A & B subgroups, a HER2-positive subgroup, an ERα- and Her2-negative subgroup, and a basal-like subgroup in which tumors are generally triple negative for ER/PR/HER2 (21–23). Significantly, BRCA1 mutant tumors were shown to cluster with the ERα-negative basal-like subgroup that display the worst overall prognosis (23).

E2 regulation of BRCA1 expression. The most potent and abundant estrogen found in women is estriol (E2); however, oestrone (E1) and oestriol (E3) also circulate throughout the body. They exert their effects by binding to the estrogen receptors, ERα and ERβ, both ligand-activated transcription factors. ERα is thought to be the most important in breast cancer development, and is a predictive marker for antiestrogen response in the clinic. The rest of this review will therefore focus on ERα.

Initial evidence to suggest interplay between ERα and BRCA1 came from mice studies, which, showed that BRCA1 levels increase dramatically during puberty and pregnancy when E2 levels increase. In addition, expression of BRCA1 was shown to be induced after treatment of ovariectomized animals with E2 (24, 25). The mechanism through which E2 regulates BRCA1 mRNA expression however has been more contentious.

Early studies suggested that E2 regulation of BRCA1 was indirect based on the delayed kinetics of induction and the fact that induction could be blocked by cycloheximide, indicating
that new protein synthesis was required (19, 20). A more recent study however showed an alternative model of regulation whereby ERα and its cofactor p300 are recruited to an activator protein site on the BRCA1 promoter after E2 stimulation (26). Subsequent studies showed that E2 stimulation of BRCA1 mRNA expression was also dependent on occupancy of the BRCA1 promoter by the unliganded aromatic hydrocarbon receptor in complex with ligand-bound ERα (Fig. 1; ref. 27). Although there are sequences resembling estrogen-responsive elements (ERE) on the BRCA1 promoter, they may not be directly responsive to E2 stimulation. It seems likely that E2 regulation of BRCA1 mRNA expression is highly complex involving a variety of ERα cofactors that may compete for ERα binding or indeed for BRCA1 promoter occupancy. The biological significance of the coordinated induction of BRCA1 expression after E2 stimulation is not yet clear but it may reflect a feedback mechanism required to control the proliferative effects of estrogens and, as such, may provide one explanation for the tissue specificity observed in BRCA1 linked tumors.

**BRCA1 regulation of ERα signaling.** Consistent with the concept that BRCA1 may function as part of a feedback mechanism to regulate estrogen signaling, BRCA1 was shown to interact with and inhibit ERα-mediated transactivation after estrogen stimulation. Specifically, it was shown that cotransfection of wild-type BRCA1 with ERα blocked the ability of ERα to transactivate reporter constructs under the control of estrogen-responsive elements. In contrast, most cancer-associated mutations of BRCA1 lack the ability to repress ERα signaling (28). This was an important finding as it suggested that BRCA1 could function as a brake on ERα-driven proliferation and showed that BRCA1 mutation released this brake. Consistent with this, it was reported that BRCA1 could abrogate the induction of over 90% of known E2-inducible genes (29). Initial studies suggested that BRCA1 functioned to block ERα transactivation after estrogen stimulation; however, BRCA1 was also shown to block ligand-independent ERα-mediated transcriptional activity (30). The mechanism through which BRCA1 inhibits ERα-mediated transcriptional activity is postulated to occur through an estrogen-independent interaction between the NH2 terminus of BRCA1 and the COOH-terminal activation domain (AF-2) with the COOH terminus of BRCA1 suggested to function as a transcriptional repression domain (31). It was subsequently shown that BRCA1 may affect ERα transcriptional activation by deregulation of p300, a well-characterized ERα coactivator. Indeed, it was shown that BRCA1 and p300 are likely to compete for the same binding site on ERα and that overexpression of p300 could reverse BRCA1-mediated repression of ERα (32). Interestingly, Cyclin D has also been reported to compete with BRCA1 for ERα binding and to reverse BRCA1-mediated repression of ERα transactivation (Fig. 1). It is worth considering the consequence of BRCA1-mediated repression of ERα signaling. ERα regulates a complex network of pathways essential for the proliferation and differentiation of both breast and ovarian tissue. The direct role played by BRCA1 in the repression of ERα-mediated transcription would be expected to attenuate the proliferative capacity of estrogens. For example, the transcriptional activation and secretion of vascular endothelial growth factor (VEGF), an estrogen-inducible gene implicated in tumor growth and angiogenesis, is severely impaired by functional BRCA1 (33).

**BRCA1 transcriptionally regulates ERα.** One may presume from the data above that loss of BRCA1 function would promote increased ERα signaling, resulting in increased proliferation and potentially malignant transformation. However, as mentioned above, the majority of BRCA1 mutant tumors do not express ERα (16, 34, 35). We recently presented data to explain this apparent paradox and provided a model to explain the high percentage of ERα deficiency observed in...
BRCA1-linked tumors. In a further twist to the story, we showed that BRCA1 could also transcriptionally induce ERα mRNA expression (36). The ability of BRCA1 to induce expression of ERα was dependent on the transcription factor Oct-1, which was required to recruit BRCA1 to the ERα promoter. Interestingly, ERα itself was not required, although ERα has been shown to autoregulate at the mRNA level. As part of the study, we showed that the BRCA1 mutant, ERα-deficient cell line HCC1937 became ERα positive after reconstitution of these cells with exogenous wild-type BRCA1. Similarly, it was shown that inactivation of endogenous BRCA1 in T47D or MCF7 cells using a siRNA approach resulted in a loss of endogenous ERα expression. Finally, we showed that inhibition of endogenous BRCA1 rendered both T47D and MCF7 cells resistant to the antiestrogen fulvestrant, an effect that could be rescued by overexpression of exogenous ERα. We therefore proposed a model whereby both alleles of BRCA1 are lost through mutation and subsequent loss of heterozygosity at a relatively early stage in BRCA1-linked breast and ovarian cancers; this has the added effect of transitioning cells from an ERα-positive to an ERα-negative genotype. Because ERα plays a central role in maintaining the luminal phenotype, these data may help explain in part the wider link between BRCA1 deficiency and the basal-like subtype of breast cancer. This is consistent with the recent report that BRCA1 expression was required for the differentiation of ERα-negative progenitor cells to ERα-positive luminal cells. The report also showed that inhibition of endogenous BRCA1 in primary breast epithelial cells led to an increase in the number of cells expressing the progenitor cell marker ALDH1 and a reduction in the number of cells expressing luminal epithelial markers and ERα (37). Taken together, these data provide a potential explanation for the distinctive histopathologic phenotype of BRCA1 mutant tumors. Interestingly, there is some indication that a proportion of sporadic basal breast cancer tumors arising in non-BRCA1 mutation carriers may actually have underlying defects in BRCA1 function, which may account for their basal phenotype (38).

**Clinical-Translational Advances**

**Can we target ERα for cancer prevention in BRCA1 mutation carriers?** In the absence of better cancer preventative measures, patients who carry a BRCA1 mutation are often offered prophylactic surgical removal of ovarian and breast tissue (39–41). A less severe primary preventative strategy such as an oral medication is highly desirable. In the case of sporadic breast cancer, tamoxifen has been shown to reduce the risk of breast cancer by ~50% (42–44). BRCA1 mutation carriers, however, do not seem to receive the same degree of protection (45). From these data, it would seem that BRCA1-linked cancers arise in a hormonally independent manner. In contrast however, removal of ovarian tissue in premenopausal BRCA1 mutation carriers has been shown to reduce the risk of breast cancer by approximately half, clearly implicating estrogen in breast cancer development (46–48). How then can these seemingly paradoxical observations be explained in light of our current understanding of BRCA1 and ERα function?

Preclinical models indicate that loss of BRCA1 function is accompanied by a loss of ERα expression. Assuming that loss of heterozygosity at the BRCA1 locus is a relatively early event in carcinogenesis, it would be expected that ERα would also be lost and the developing tumor would be hormonally independent (Fig. 2). This would explain the failure of tamoxifen as a chemopreventative agent in these patients. Why then does oophorectomy protect against breast cancer in these patients? One possible explanation is that estrogen metabolites can be genotoxic in their own right. This hypothesis has been supported by a number of epidemiologic studies that have confirmed the carcinogenic effect of prolonged exposure to estrogens (49, 50). The reaction of specific estrogen metabolites such as catechol estrogens-3-4quinones with DNA results in the formation of depurinating adducts that are mutagenic (44). It is possible that the consequent accumulation of mutagenic metabolites in ERα-responsive tissues such as breast increases the statistical likelihood of losing the second BRCA1 allele in BRCA1 mutant carriers thereby initiating tumor formation. Oophorectomy reduces the levels of estrogen in premenopausal women, thereby reducing the levels of genotoxic metabolites.
Tamoxifen however, does not reduce estrogen levels and would not be predicted to protect against cancer in mutation carriers, as is observed in the clinic. Effective breast cancer prophylaxis may therefore require ovarian suppression either through surgical resection or through the administration of gonadotrophin antagonists such as goserelin. However, it is important to note that BRCA1 mutant carriers are at risk of ovarian cancer and in the absence of an effective prophylactic approach, removal of the ovaries would still be preferable. In postmenopausal women, the primary source of estrogen is generated through the aromatase pathway in adipose and muscle tissue (51). It is possible therefore that unlike tamoxifen, aromatase inhibitors, such as anastrozole, may be effective as a breast cancer preventative agent in BRCA1 mutation carriers who have undergone natural or surgical menopause as these agents block production of estrogen and thereby are likely to prevent production of secondary carcinogenic metabolites.

Conclusions. The continuing investigation of the complex relationship between BRCA1 and ERα has provided potential answers to help understand some of the important clinical facts, such as the ERα deficiency observed in BRCA1-linked breast cancer. In addition to the potential ability of estrogen metabolites to induce loss of the second BRCA1 allele, it has also been suggested that estrogen may somehow facilitate the survival of BRCA1 deficient cells in hormonally responsive tissue. Although this may be a reasonable hypothesis for BRCA2-linked breast cancers, it is unlikely to be the case for BRCA1-linked cancers as they are ERα deficient and unlikely to gain a selective proliferative advantage from estrogen. Another possibility is that BRCA1 may function as a specific regulator of cell fate in hormonally responsive tissues. Loss of BRCA1 may result in the dedifferentiation of cells toward a more resilient basal/stem cell–like genotype. These dedifferentiation breast cells may be capable of surviving the genomic instability caused by loss of BRCA1 potentially by selecting for concurrent p53 loss as is observed in the majority of BRCA1-deficient tumors. Although the underlying molecular basis for the tissue specificity observed for BRCA1-linked tumors still remains to be resolved, it is likely to be highly complex and dependent on known/unknown functions of both BRCA1 and ERα.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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