BRCA1 in hormonal carcinogenesis: basic and clinical research

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Abstract

The breast and ovarian cancer susceptibility gene-1 (BRCA1) located on chromosome 17q21 encodes a tumor suppressor gene that functions, in part, as a caretaker gene in preserving chromosomal stability. The observation that most BRCA1 mutant breast cancers are hormone receptor negative has led some to question whether hormonal factors contribute to the etiology of BRCA1-mutant breast cancers. Nevertheless, the caretaker function of BRCA1 is a generic one and does not explain why BRCA1 mutations confer a specific risk for tumor types that are hormone-responsive or that hormonal factors contribute to the etiology, including those of the breast, uterus, cervix, and prostate. An accumulating body of research indicates that in addition to its well-established roles in regulation of the DNA damage response, the BRCA1 protein interacts with steroid hormone receptors (estrogen receptor (ER-α) and androgen receptor (AR)) and regulates their activity, inhibiting ER-α activity and stimulating AR activity. The ability of BRCA1 to regulate steroid hormone action is consistent with clinical-epidemiological research suggesting that: (i) hormonal factors contribute to breast cancer risk in BRCA1 mutation carriers; and (ii) the spectrum of risk-modifying effects of hormonal factors in BRCA1 carriers is not identical to that observed in the general population. These data suggest a model for BRCA1 carcinogenesis in which genomic instability leads to the initiation of cancerous cell clones, while loss of normal restraint on hormonal stimulation of mammary epithelial cell proliferation allows amplification of these pre-existing clones. Further research will be required to substantiate this hypothesis.

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Introduction

The first breast cancer susceptibility gene, BRCA1, was identified and cloned in 1994 by Miki and colleagues after an intensive search (Miki et al. 1994). A year later, a second breast cancer susceptibility gene, BRCA2 was identified (Wooster et al. 1995, Tavtigian et al. 1996). Mutations of BRCA1 or BRCA2 account for most hereditary breast cancer and breast plus ovarian cancer families, although there are a sufficient number of non-BRCA1/BRCA2 breast cancer families to suggest the existence of at least one additional BRCA gene. A recent study of 11 847 individuals from 699 BRCA1 mutant breast cancer families suggests that in addition to breast and ovarian cancers, BRCA1 mutation carriers have a significantly increased risk of pancreatic, endometrial, and cervical cancers and for prostatic cancers in men younger than age 65 (Thompson et al. 2002). Breast, endometrial, and prostate cancers are all known to be steroid hormone-responsive tumor types; the first two are estrogen-responsive, while the third is androgen-responsive. In addition, there is evidence supporting a role for estrogen in stimulating cell growth during the pathogenesis of cervical cancer in animal models and in humans (Elsen et al. 2000, Arbe et al. 2003, de Villiers 2003, Li et al. 2003) although the hormonal etiology of cervical cancer is not as well established as in the other tumor types. BRCA2 mutations have been linked to ovarian, pancreatic, and prostatic cancers; but unlike BRCA1, BRCA2 mutations have not been linked to cervical and endometrial cancers (Streuwng et al. 1997, Liede et al. 2004, Lubinski et al. 2004). These findings suggest that endocrine factors may contribute to BRCA1-dependent cancer development.
BRCA1 fulfills the criteria for a tumor suppressor gene (TSG), since the vast majority of cancers that develop in mutation carriers exhibit loss of the wild-type allele (Merajver et al. 1995). This consideration implies that its normal function is required to block tumor development. During the 10 years since its discovery, a variety of studies have established functional roles for BRCA1 in DNA damage signaling, several different DNA repair processes, apoptosis susceptibility, and several DNA damage-responsive cell cycle checkpoints (Venkitaraman 2002, Rosen et al. 2003). Consistent with these functional roles, cultured cells and tumors deficient for BRCA1 exhibit a pattern of severe genomic instability, characterized by aneuploidy, centrosomal amplification, and chromosomal aberrations (Tirkkonen et al. 1997, Xu et al. 1999a, Weaver et al. 2002). Taken together, these data suggest that BRCA1 may function as a ‘caretaker’ in preserving the integrity of the genome. Loss of this caretaker function may explain why BRCA1 mutations predispose to the development of cancer, but it does not by itself explain the predilection to specific tumor types, particularly breast cancer and other hormone-dependent tumors. In order to elucidate the reasons that BRCA1 mutations confer risk for hormone-responsive cancers, this review will cover basic science aspects of BRCA1: hormone interactions and clinical-epidemiological aspects of this topic.

Basic research

The BRCA1 gene and protein

The human BRCA1 gene occupies a region of about 100 kilobases on chromosome 17q21 and contains 24 exons, 22 of which are coding exons and two of which are non-coding (Miki et al. 1994). The BRCA1 protein does not exhibit any significant structural homology to other known proteins except for the presence of a conserved N-terminal RING finger domain (amino acid 20–64) and a C-terminal acidic domain capable of mediating transcriptional activation when linked to a suitable DNA-binding domain (Miki et al. 1994, Monteiro et al. 1996). The N-terminal RING domain of BRCA1 interacts with another RING finger protein, BARD1 (BRCA1-associated ring domain protein 1) and the BRCA1:BARD1 complex mediates a ubiquitin ligase activity that may be important for tumor suppression (Hashizume et al. 2001). The C-terminal transcriptional activation domain (TAD) of BRCA1 contains a tandem repeat of 95 amino acids called a BRCA1-associated C-terminal domain (BRCT) that is homologous to similar domains contained in various DNA repair and cell cycle checkpoint proteins (Bork et al. 1997). The BRCA1 protein contains functional nuclear import and nuclear export signals, suggesting that it may shuttle back and forth between the nucleus and cytoplasm, although it appears that most, if not all, BRCA1 functions occur within the nucleus (Thakur et al. 1997, Rodriguez & Henderson 2000).

The BRCA1 protein is a 220-kDa (predominantly) nuclear phosphoprotein that is expressed and phosphorylated in a cyclic fashion, with maximal expression/phosphorylation in late G1 and early to mid S-phase (Rajan et al. 1996, Thomas et al. 1997). It was originally thought that BRCA1 expression is regulated by estrogen through an estrogen response element (ERE)-like sequence in its promoter. However, it was subsequently demonstrated that the induction of BRCA1 expression by estrogen in estrogen-responsive human breast cancer cells occurs indirectly, due to entry into S-phase (Marks et al. 1997). Many, if not most of BRCA1’s biologic actions are mediated through regulation of transcription. While BRCA1 is not a sequence-specific DNA binding transcription factor, it can interact with a variety of transcription factors and either stimulate or inhibit their activity (e.g. p53, STAT1, c-Myc, JunB, ATF-1, and others) (Rosen et al. 2003). BRCA1 can also interact with components of the basal transcriptional machinery (e.g. RNA helicase A, RNA polymerase II), transcriptional coregulators and chromatin-modifying proteins (e.g. p300/CBP, the retinoblastoma protein (RB1), several RB-associated proteins (RbAp46 and RbAp48), histone deacetylases (HDACs), the SWI/SNF-related transcriptional activator BRG1, the cofactor of BRCA1 (COBRA1)), and other transcriptional regulatory proteins (Rosen et al. 2003).

BRCA1 regulation of steroid hormone receptor action

Estrogen receptor (ER-α)

A molecular linkage between BRCA1 and estrogen action was established by the observation that exogenous BRCA1 inhibits the transcriptional activity of the liganded ER-α through the ERE in cultured human breast and prostate cancer cell lines (Fan et al. 1999). Transcriptional assays revealed that BRCA1 blocked the activity of the intact ER-α and that of the conserved C-terminal activation domain of ER-α (AF-2; which is linked to the ligand-binding domain) but did not inhibit the constitutively active N-terminal activation domain (AF-1) (Fan et al. 1999, 2002). BRCA1 also blocked the 17β-estradiol (E2)-stimulated
expression of two estrogen-responsive genes, pS2 and cathepsin D (Fan et al. 2001a).

Further studies suggest two mechanisms for BRCA1 repression of ER-α: (i) a direct interaction of the BRCA1 and ER-α proteins; and (ii) down-regulation of p300, a nuclear receptor coactivator (Fan et al. 1998, 2001a,b, 2002, Kawai et al. 2002). The BRCA1: ER-α interaction was mapped to the N-terminus of BRCA1 and AF-2 domain of ER-α and did not require the presence of E2 (Fan et al. 2001a). In contrast to wild-type BRCA1 (wtBRCA1), a series of truncated BRCA1 proteins and cancer-associated mutants failed to, or showed reduced ability to, repress ER-α activity, consistent with the idea that ER-α repression contributes to BRCA1’s tumor suppressor function.

As noted above, the BRCA1 N-terminal RING domain was found to interact with the BRCA1-associated RING domain protein 1 (BARD1) protein (Wu et al. 1996) through a RING: RING interaction (Brzovic et al. 2001) and the RING heterodimer possesses strong ubiquitin ligase activity (Hashizume et al. 2001). It has been proposed that this ubiquitin ligase activity is required for BRCA1 tumor suppressor function; and it has been shown that the ubiquitin ligase activity is required for the function of BRCA1 in maintaining the normal state of cellular radiation resistance (Ruffner et al. 2001). With regard to ER-α activity, the RING domain of BRCA1 is not required for the physical interaction of BRCA1 with ER-α but is required for BRCA1 repression of ER-α activity, since two cancer-associated full-length BRCA1 point mutants that disrupt the RING domain structure (61Cys—Gly and 64Cys—Gly) failed to inhibit ER-α transcriptional activity (Fan et al. 2001a). The mechanism by which these RING domain mutations abrogate the ability of BRCA1 to repress ER-α activity is unclear. It could be through disruption of the ubiquitin ligase activity or another function of the RING. Although there is no evidence that BRCA1 causes the degradation of ER-α, few targets of its ubiquitin ligase have been identified; and the possibility that one of these targets could contribute to the inhibition of ER-α is not ruled out.

wtBRCA1 down-regulated the expression of p300 but not its functional homolog, the CREB binding protein (CBP) (Fan et al. 1998, 2002). Exogenous p300 or CBP rescued the wtBRCA1-mediated repression of ER-α activity and the rescue activity mapped to a conserved cysteine-histidine rich region (CH3), that was necessary and sufficient for rescue (Fan et al. 2002). Interestingly, a direct interaction between the CH3 domain of p300/CBP and the AF-2 domain of ER-α was documented, suggesting that BRCA1 and p300/CBP may compete for binding to AF-2. Finally, the ability of p300 mutants missing the histone acetyltransferase (HAT) or steroid receptor coactivator-1 (SRC-1) binding domains to rescue the BRCA1 repression of ER-α, coupled with the inability of co-activators glucocorticoid receptor interacting protein-1 (GRIP1) and p300/CBP-associated factor (PCAF) to rescue repression suggest that derepression and co-activation of ER-α are distinct actions with different structural requirements.

A more detailed study of the BRCA1:ER-α interaction revealed two potential contact sites for BRCA1 on ER-α (the major site within amino acids 338–379 and a minor site within amino acids 420–595) and two contact sites for ER-α on BRCA1 (amino acids 67–100 and 101–134) (Ma et al. 2005). BRCA1 contains a conserved helical motif (amino acids 86–95) resembling a previously identified nuclear corepressor motif (Lxx(I/H)xnx(1/L), where x = any amino acid), mutation of which disrupted the ability of BRCA1 to bind and repress ER-α (Ma et al. 2005). Based on these studies, a partial BRCA1: ER-α three-dimensional structure was proposed (Fig. 1). In this computer-generated model, BRCA1 heterodimerizes with ER-α via the anti-parallel α-helix domain, mainly using the third helix (amino acids 80–96) of BRCA1. The ER-α side of the interacting surface is an α-helix of ER-α (amino acids 338–379), which is at the opposite side of the ER-α homodimerization interface. Interestingly, two tumor-associated BRCA1 mutations at the interacting surface (L63F and I89T) were found to impair the ability of wtBRCA1 to repress ER-α activity (Ma et al. 2005).

Zheng and colleagues (2001) demonstrated constitutive activation of exogenous ER-α in the Brca1-null mouse embryo fibroblasts (MEFs), suggesting that the endogenous BRCA1 protein may mediate the ligand-independent repression of ER-α. Consistent with such a role for BRCA1, the same investigators also showed that BRCA1 was present at the ERE site on the promoter of the estrogen-responsive gene cathepsin D in MCF-7 human breast cancer cells before but not after stimulation with E2. Consistent with these findings, knockdown of endogenous BRCA1 by RNA interference conferred ER-α activation in MCF-7 cells in the absence of E2 (Jones et al. 2005). Furthermore, BRCA1 knockdown enhanced the degree of E2-stimulated ER-α activity, with a higher-fold stimulation of ER-α activity at lower doses of E2. These findings suggest that the absence or inactivation of BRCA1 may allow ER-α activation under physiologic conditions of low levels or no E2.

A second estrogen receptor, ER-β, is similar in structure to ER-α, but exhibits a different tissue
distribution and both similar and distinct functional properties relative to ligand selectivity, binding affinity, and transcriptional activation (Mosselman et al. 1996). Several studies suggest that ER-β may inhibit ER-α activity (e.g. in the uterus) and the inhibition is due, in part, to the estrogen-inducible formation of ER-α/ER-β (Pettersson et al. 2000, Weihua et al. 2000, Jisa & Jungbauer 2003). The co-expression of ER-α and ER-β not only conferred a reduced sensitivity to estrogen but also caused a decrease in the agonist activity and an increase in the antagonist activity of tamoxifen (Hall & McDonnell 1999, Pettersson et al. 2000). Whether ER-β contributes to the ability of BRCA1 to inhibit ER-α activity is unknown. However, it has been recently shown that knockdown of endogenous BRCA1 levels by RNA interference enhances the agonist activity of tamoxifen (Jones et al. 2005). The possibility of a functional interaction between BRCA1 and ER-β warrants study.

Recent work suggests the existence of a pool of ER-α that is localized at the plasma membrane, G-protein coupled, and mediates signaling, in part, through cross-talk with the epidermal growth factor receptor (EGFR) or insulin-like growth factor-1 receptor (IGF1R) (Kelly & Levin 2001). Razandi and colleagues (2004) found that in the estrogen-responsive breast cancer cell lines MCF-7 and ZR-75-1, E2 caused a rapid and sustained activation of extracellular signal-related kinase (ERK) signaling that was substantially blocked by wild-type but not mutant BRCA1 (Razandi et al. 2004). BRCA1 blocked EGF-induced ERK activation and cell proliferation through a mechanism that involves an ERK phosphatase, mitogen-activated kinase phosphatase 1. These findings suggest that BRCA1 may inhibit E2-stimulated cell proliferation, in part, by inhibiting cross talk with growth factor receptors.

Androgen receptor (AR)

Androgen receptor signaling plays a major role in human prostate carcinogenesis (Henderson & Feigelson 2000). In several studies, BRCA1 was found to interact directly with AR and stimulate its activity.
(Park et al. 2000, Yeh et al. 2000). BRCA1 up-regulated the AR-mediated expression of the G1 cell cycle inhibitor p21\textsuperscript{WAF1} and enhanced dihydrotestosterone (DHT)-induced cell death in human prostate cancer cells (Yeh et al. 2000). BRCA1 was also found to interact directly with both AR and the coactivator GRIP1 and to stimulate AR activity via the activation factor (AF-1) domain of AR (Park et al. 2000). The ability of BRCA1 to stimulate AR activity was enhanced by several coactivators, including CBP, ARA55, ARA70 and GRIP1.

AR mutations have been linked to the development of male breast cancer, and androgens can inhibit the proliferation of breast cancer cells and block E2-stimulated proliferation, suggesting a potential role for the AR in mammary carcinogenesis (Ando et al. 2002). The AR exhibits genetic polymorphism in the number of polyglutamine (CAG) repeats in its AF-1 domain, with the repeat length inversely correlated with p160 coactivator binding and AR activity. Some studies suggest an association between a long CAG repeat length and an early age of breast cancer onset in BRCA1 mutation carriers, while other do not show such a correlation (Ferro et al. 2002).

**Regulation of BRCA1 expression by agents that regulate steroid hormone receptor action**

Indole-3-carbinol (I3C) is a micronutrient found in cruciferous vegetables (e.g. cabbage) with cancer preventive activity, particularly for E2-dependent cancers (breast, cervical and endometrial; Shertzer & Senft 2000). Diets rich in these vegetables have been linked to a decreased risk of breast cancer; and dietary supplementation with cruciferous vegetables or with I3C itself blocks the formation of E2-dependent tumors in animals. I3C-mediated protection against mammary carcinogenesis is thought to be due, in part, to stimulation of metabolism of estrone through the 2-hydroxylation pathway, which yields inactive products, at the expense of 16-hydroxylation, which yields potentially carcinogenic metabolites (Bradlow et al. 1991). Recently, it was found that I3C and its major active metabolite diindolylmethane (DIM) up-regulate BRCA1 mRNA and protein expression in breast and cervical cancer cell lines (Meng et al. 2000a,b, Carter et al. 2002). It was also found that I3C inhibits ER-\(\alpha\) activity, raising the possibility that BRCA1 mediates some of its actions. Genistein, an isoflavonoid derived from soy with cancer prevention activity, can also up-regulate BRCA1 expression (see below).

Moderate alcohol consumption is a risk factor for breast cancer and synergistically enhances risk in combination with estrogen replacement therapy (Holmes & Willett 2004). The molecular basis for this increased risk is unclear. A recent study revealed that exposure of breast cancer cells to ethanol stimulates cell migration and invasion and enhances ER-\(\alpha\) signaling (Fan et al. 2000). In addition to causing a modest increase in ER-\(\alpha\) protein levels, ethanol caused a large dose-dependent decrease in BRCA1 levels, suggesting that BRCA1 loss may contribute to increased ER-\(\alpha\) signaling.

Persistent organochlorines (POCs) are carcinogens that contaminate the food chain. Some POCs inhibit ER-\(\alpha\) activity and may contribute to breast cancer risk. Polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (toxiphen) were found to down-regulate E2-stimulated BRCA1 mRNA expression in MCF-7 breast cancer cells (Rattenborg et al. 2002). The polycyclic aromatic hydrocarbon benzo(a)pyrene, a carcinogen, down-regulated BRCA1 expression in ER-\(\alpha\) positive but not ER-\(\alpha\) negative breast cancer cell lines (Jeffy et al. 2002). These findings suggest that BRCA1 may be a molecular target for both cancer prevention agents and carcinogens.

**Animal Studies**

**Role of BRCA1 in development and mammary carcinogenesis**

Several different studies showed that BRCA1 null mutations in mice lead to early embryonic lethality (day 6.5 to 9.5; Gowen et al. 1996, Hakem et al. 1996, Liu et al. 1996). The BRCA1 null phenotype was characterized by widespread defects in cell proliferation due, in part, to p53 activation; and the phenotype was partially rescued by a p53 or p21WAF1 deficiency (Hakem et al. 1997). Studies of the patterns of BRCA1 mRNA expression revealed that BRCA1 is highly expressed in multiple tissues, in rapidly proliferating cells undergoing differentiation, including mammary epithelial cells during puberty, pregnancy and lactation (Lane et al. 1995, Marquis et al. 1995). Consistent with these findings, BRCA1 expression was up-regulated in cultured mammary epithelial cells induced to differentiate (Rajan et al. 1996) and wtBRCA1 caused accelerated differentiation independently of its effects on cell proliferation and p53 transactivation, suggesting a role for BRCA1 in mediating differentiation (Kubista et al. 2002).

BRCA1 was expressed in the granulosa and theca cells of the mouse ovary independently of hormonal stimulation (Phillips et al. 1997). The pattern of expression of BRCA2 was generally similar to BRCA1;
but differential expression was observed in endocrine tissues, including the testis during spermatogenesis and the breast during pregnancy (Rajan et al. 1997). In studies of the effect of a synthetic estrogen (diethylstilbestrol (DES)) on endocrine responses in Brca1 heterozygous mice, mammary ductal branching was reduced in DES-treated heterozygotes, relative to wild-type mice (Bennett et al. 2000). Most heterozygous mice showed ovarian atrophy, as compared with wild-type mice which showed arrested follicular development. These findings suggest that Brca1 may be haplo-insufficient for mediating some proliferative endocrine tissue responses to DES.

Chuxia Deng and his colleagues developed a mouse model featuring a mammary-targeted homozygous deletion of Brca1 exon 11 (which codes for >60% of the Brca1 protein). These mice developed mammary cancers after a latent period of 12 months, which was significantly reduced in the setting of a heterozygous p53 deletion (Xu et al. 1999b). Mammary tumors from these mice recapitulated some of the features of BRCA1 mutant human breast cancers (e.g. chromosomal rearrangements, p53 mutations, and ER-α negativity) but not others (e.g. absence of HER2/Neu or cyclin D1 over-expression (see below)) (Brodie et al. 2001, Deng 2002).

A study of the effect of tamoxifen, a selective estrogen receptor modulator, in the same model revealed tamoxifen actually increased the incidence of mammary cancers in Brca1-deficient animals (Jones et al. 2005). Consistent with this finding, knockdown of endogenous BRCA1 enhanced the ER-α agonist activity of tamoxifen in cultured MCF-7 cells (Jones et al. 2005). Tamoxifen-induced mammary hyperplasias in the Brca1-deficient mice showed a loss of ER-α expression, suggesting that early loss of ER-α is a feature of the pathogenesis of these tumors. These findings suggest that hormonal factors can modify development of Brca1 mutant mammary cancers in this model.

Interestingly, a prepubertal (age 1–3 weeks) exposure of female rats to 17β-estradiol or genistein (a phytoestrogen with mixed agonist/antagonist properties) decreased the subsequent risk of developing 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors (Cabanes et al. 2004). These exposures caused a persistent up-regulation of BRCA1 expression in the mammary glands that was still observed at age 16 weeks. While genistein can activate ER-α by itself and inhibit estradiol-stimulated ER-α activity, it appears to be a more selective ligand for ER-β than for ER-α since it competes more effectively with estradiol for binding to ER-β than to ER-α (Barkhem et al. 1998, Kuiper et al. 1998, Pike et al. 1999). Whether the ability of genistein to up-regulate Brca1 expression is dependent upon ER-β remains to be determined. As noted earlier, BRCA1 is normally highly expressed in proliferating mammary epithelial cells undergoing differentiation during puberty and pregnancy in mice. Taken together, these findings suggest that the expression of BRCA1 during key windows of time when the mammary gland is hormonally sensitive may be critical for tumor suppression.

Clinical research

Epidemiology of BRCA1 mutant cancers

Clinicopathologic characteristics of BRCA1 mutant breast cancers

By age 70 the risk of breast cancer in BRCA1 mutation carriers is estimated to be between 55 and 85%, while the risk of ovarian cancer is approximately 40% (Easton et al. 1995, Streuwing et al. 1997, Warner et al. 1999, Antoniou et al. 2003). These cancers typically occur at a younger age than is seen in the general population, with studies demonstrating that approximately one third to one half of mutation carriers will be diagnosed with breast cancer by age 50 (Easton et al. 1995, Antoniou et al. 2003). The breast cancers diagnosed in BRCA1 carriers differ histopathologically from tumors that occur in women with sporadic disease. These tumors are usually hormone receptor negative, with different studies indicating that only 10 to 36% of tumors in these patients are estrogen receptor positive (Verhoog et al. 1998, Lakhani et al. 2002, Robson et al. 2004). The ER-α negativity of these tumors may be due, in part, to hypermethylation of the ER-α gene (Archey et al. 2002). Interestingly, however, progesterone receptor (PR) expression was significantly higher in benign mammary epithelial cells adjacent to a BRCA1 mutant breast cancer than a sporadic cancer (King et al. 2004). In addition, BRCA1 mutant tumors are more frequently high grade (Breast Cancer Linkage Consortium 1997), have higher mitotic counts (Marcus et al. 1996, Breast Cancer Linkage Consortium 1997, Lakhani et al. 1998), and have medullary or atypical medullary features (Eisinger et al. 1998).

Whereas cyclin D1 amplification and/or over-expression are relatively common in sporadic breast cancers, BRCA1 mutant cancers showed no cyclin D1 amplification and low levels of cyclin D1 expression (Osin et al. 1998, Armes et al. 1999, Vaziri et al. 2001). The c-Myc oncogene is amplified in 15–20% of sporadic breast cancers. In contrast to HER2/Neu and cyclin D1, two recent studies reported an excess prevalence of c-Myc amplification in BRCA1 mutant cancers relative to sporadic cancers (Adem et al. 2004, Grushko et al. 2004), although one study did not find an excess of c-Myc amplification in BRCA1 mutant cancers (Palacios et al. 2004). Unlike sporadic breast cancers, BRCA1 mutant tumors showed frequent amplification of c-Myb, a proto-oncogene implicated in mediating cell cycle progression from G2 to M (Kauraniemi et al. 2000). In addition to ER-α and the progesterone receptor, BRCA1 mutant breast cancers also show reduced AR expression, as compared with sporadic cancers (Berns et al. 2003). DNA microarray and immunohistochemistry based studies indicate that breast cancers in BRCA1 carriers exhibit a basal-like phenotype (Foulkes et al. 2003, Sorlie et al. 2003). The basal phenotype, which occurs in about 15% of breast cancers, is characterized by ER-α and HER2/Neu negativity, coupled to the expression of high levels of the cytokeratins normally found in basal epithelial cells.

Recent studies indicate that BRCA1 mRNA and protein are absent or significantly reduced in 30–40% of sporadic breast cancer cases (Ozcelik et al. 1998, Taylor et al. 1998, Rio et al. 1999, Wilson et al. 1999). Decreased BRCA1 expression may be due to hypermethylation of the BRCA1 promoter on CpG islands and/or loss of one of the BRCA1 alleles (Rice et al. 1998, Esteller et al. 2000, Staff et al. 2003). It is not yet clear whether these BRCA1 under-expressing breast cancers represent a distinct phenotypic group. However, since most sporadic breast cancers are ER-α positive, these findings suggest a role for a BRCA1: hormone interaction in sporadic breast cancer development.

**Hormonal modifiers of cancer risk**

Given the differences in BRCA1 mutant breast cancers, particularly their preponderance toward hormone receptor negativity, the significance of endocrine factors in the development and prevention of these cancers has been questioned. The study of cancer risk modifiers provides important clues as to the impact of hormone related factors on the development of breast cancer in mutation carriers. In the general population, endocrine factors, such as age at menarche, age at menopause, age at the first full-term pregnancy, parity, breast feeding and the use of hormone replacement therapy impact breast cancer risk. Various studies have explored the role of some of these factors in BRCA1 carriers and reveal that a number of them modify breast cancer risk.

**Parity and age at first full-term pregnancy**

Early age at first full-term pregnancy and increasing parity reduce the risk of breast cancer in the general population. However, data on the effect of these factors in mutation carriers have yielded different results, with some studies indicating no impact on cancer risk and other studies suggesting that late age at first full-term pregnancy confers protection against breast cancer and that increasing parity increases the risk of this disease. In a community-based study of over 5000 Ashkenazi Jewish women from Washington DC, women who did not carry founder mutations in BRCA1 or BRCA2 had about a 5% increase in risk of breast cancer with each 5 year increase in age at first birth (relative risk (RR) per 5 years = 1.05; 95% confidence interval (CI) = 0.96 to 1.15) whereas mutation carriers had a 35% decrease in risk with each 5 year increase in age at first birth (RR per 5 years = 0.65; 95% CI = 0.35 to 1.12; Hartge et al. 2002). A case control study, which included 685 BRCA1 carriers and 280 BRCA2 carriers with breast cancer and matched carrier controls, showed no difference in age at first full-term pregnancy in those with and without breast cancer (Jernstrom et al. 2004). With respect to parity, this study revealed no difference in mean number of pregnancies between mutation carriers with and without breast cancer. An earlier study from the same group suggested that the risk of breast cancer prior to age 40 in BRCA1/2 carriers increased with increasing number of births (Jernstrom et al. 1999). While the findings from this study are provocative, the number of participants was small and likely included some of the same women as were included in the later larger analysis that showed no association between parity and breast cancer risk (Jernstrom et al. 2004). Taken together, these studies suggest that the usual protective effect of early age at first birth and increasing parity is not observed in mutation carriers.

**Age at menarche**

While the impact of age at menarche in BRCA mutation carriers was not specifically addressed, one case control study noted a very small but statistically
significant difference in age at menarche in BRCA1/2 mutation carriers with breast cancer and those unaffected by this disease (12.8 vs 12.9 years, \( P = 0.03 \); Jernstrom et al. 2004). However, the majority of studies have found no significant differences in age at menarche amongst BRCA1 carriers who developed breast cancer as compared with those who had not (Jernstrom et al. 1999, Rebbeck et al. 2001, 2002). Thus, in contradistinction to what is observed in the general population, these studies have not demonstrated that early age at menarche influences breast cancer risk in mutation carriers.

**Breast feeding**

Similar to observations in the general population, prolonged breast feeding has been shown to have a protective effect in BRCA1 carriers. A recently published case-control study revealed that BRCA1 carriers who breast fed for more than one year were significantly less likely to develop breast cancer than were BRCA1 carriers who never breast fed (odds ratio = 0.55; 95% CI = 0.38 to 0.80, \( P = 0.001 \); Jernstrom et al. 2004). To control for the close relationship between duration of breast feeding and parity, nulliparous women were excluded from the analysis and cases and controls were matched for parity. It has been reported that BRCA1 carriers may experience difficulties with milk production, raising the possibility that this factor, rather than the duration of breast feeding, is associated with the increased risk of breast cancer seen in BRCA1 carriers who report a shorter duration of breast feeding.

**Oral contraceptive use and hormone replacement therapy (HRT)**

Although epidemiologic studies have yielded varying results, a meta-analysis of 54 studies suggests that the long-term use of oral contraceptives confers a small but significant increase in the risk of breast cancer in young women (Collaborative Group on Hormonal Factors in Breast Cancer 1996). Additionally, oral contraceptive use has also been associated with an increased risk of breast cancer in those with a first degree relative with this disease (Grabrick et al. 2000). In a case control study of BRCA1 and BRCA2 mutation carriers, BRCA1 mutation carriers who used oral contraceptives for at least five years (odds ratio = 1.33; 95% CI = 1.11 to 1.60) or those who used oral contraceptives before age 30 (odds ratio = 1.29; 95% CI = 1.09 to 1.52) had a modest but significant increase in breast cancer risk. In contrast, BRCA2 carriers did not exhibit an increased risk associated with oral contraceptive use (Narod et al. 2002). Another report describes a significant increase in breast cancer risk for BRCA1 carriers in the first 15 years after last use (hazard ratios from 1.69–1.99) but in the same study users with more than 15 years since last use showed a reduced risk (hazard ratio = 0.69; Heimdal et al. 2002). An earlier study with smaller patient numbers described an increased breast cancer risk among oral contraceptive users with either a BRCA1 or a BRCA2 mutation (Ursin et al. 1997).

In contrast to breast cancer, several studies suggest that oral contraceptive use confers a reduced risk of ovarian cancer in BRCA1 mutation carriers, the extent of which increases with increasing duration of use (McGuire et al. 2004, Whittemore et al. 2004). Oral contraceptive use is also inversely correlated with ovarian cancer risk in the general population and the magnitude of risk reduction appears to be similar for BRCA1 mutation carriers and non-carriers.

A meta-analysis of 51 epidemiologic studies on HRT in postmenopausal women revealed a modest but significant increase in risk of breast cancer associated with a prolonged duration of HRT (>5 years) within the first 5 years since last HRT use but not thereafter (Collaborative Group on Hormonal Factors in Breast Cancer 1997). More recently, data from the Women’s Health Initiative, a large randomized placebo controlled trial of estrogen plus progestin or estrogen alone indicated that using a combination of estrogen plus a progestin confers a higher risk for breast cancer than utilizing estrogen only (The Women’s Health Initiative Steering Committee 2004, Writing Group for the Women’s Health Initiative Investigators 2002). At present, there is insufficient data to ascertain whether HRT confers a significantly increased risk for breast cancer in BRCA1 mutation carriers (Narod 2001).

**Clinical Interventions**

The epidemiologic studies described above suggest that the hormonal milieu modifies the risk for breast cancer in BRCA1 mutation carriers, although the impact of specific factors on cancer risk may differ between the carriers and the general population. Studies on clinical interventions to prevent BRCA1 mutant cancers shed further light on the role of hormones in the etiology of these cancers.

**Prophylactic oophorectomy**

Studies on the effect of prophylactic oophorectomy have provided some of the most convincing evidence that hormone-related factors influence the risk of
breast cancer in mutation carriers. This procedure removes the major source of two hormones with potent effects on mammary physiology and cancer development, estrogen and progesterone. In a multi-institution study, mutation carriers who had undergone prophylactic oophorectomy (cases) were matched by type of mutation (BRCA1 vs BRCA2), age, and institution to mutation carriers who had not undergone prophylactic oophorectomy (controls) (Rebeck et al. 2002). None of these subjects had undergone prophylactic mastectomy. With a median follow-up of about 11 years, 21 of 99 cases (21%) and 60 of 142 controls (42%) had been diagnosed with breast cancer (hazard ratio = 0.47; 95% CI = 0.29 to 0.77). Similarly, in a prospective study of 170 newly identified BRCA1 or BRCA2 mutation carriers, breast cancer was diagnosed in 3 of the 69 women who chose to undergo bilateral salpingo-oophorectomy and no prophylactic mastectomy (4%) as compared with 8 of the 62 women who underwent neither prophylactic oophorectomy nor mastectomy (13%) (hazard ratio = 0.32; 95% CI = 0.08 to 1.20) (Kauff et al. 2002). While neither of these studies specifically addressed the impact of such an intervention in BRCA1 as opposed to BRCA2 carriers, the majority of women in both of these studies harbored BRCA1 mutations (83% and 61%, respectively).

Additionally, the impact of prophylactic oophorectomy on the subsequent risk of contralateral breast cancer has been examined in carriers with breast cancer. A recent prospective study of 491 BRCA1/2 carriers with stage I or II breast cancer, demonstrated a 10 year risk of contralateral disease of 29.5% (Metcalfe et al. 2004). Amongst the 336 carriers in whom the contralateral breast was intact, prophylactic oophorectomy was associated with a 59% reduction in risk of contralateral breast cancer (hazard ratio = 0.41; 95% CI = 0.18 to 0.90). The benefit of this procedure was greatest for those who underwent prophylactic oophorectomy at less than 50 years of age (hazard ratio = 0.24; 95% CI = 0.07 to 0.77) as compared with those who were 50 or greater (hazard ratio = 0.91; 95% CI = 0.26 to 3.21). In multivariate analyses restricted to the 224 BRCA1 carriers, oophorectomy was demonstrated to reduce the risk of contralateral disease (hazard ratio = 0.33; 95% CI = 0.13 to 0.84). These data support the idea that hormonal factors play a role in the development of BRCA1 mutant breast cancers.

**Tamoxifen**

The National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (NSABP-P1) examined the role of tamoxifen in reducing the risk of breast cancer in healthy women at increased risk for this disease due to a variety of predisposing factors, including family history, age, and certain high risk conditions such as lobular carcinoma in situ or atypical hyperplasia (Fisher et al. 1998). Among the whole study population in this randomized placebo-controlled trial, tamoxifen reduced the risk of invasive breast cancer by about half. Whereas tamoxifen reduced the risk of developing estrogen receptor positive tumors by 69%, no significant impact on the occurrence of estrogen receptor negative disease was appreciated. Given that BRCA1 carriers tend to develop hormone receptor negative breast cancers, significant debate exists as to the role of tamoxifen as a chemopreventive agent in these women.

In order to further address the potential effect of tamoxifen as a risk-reducing agent in unaffected women with hereditary breast cancer, genetic analysis was performed on 288 of the NSABP-P1 participants who developed breast cancer (King et al. 2001). Only 19 of these 288 women (6.6%) were found to carry disease-conferring mutations. These 19 women included 8 women with BRCA1 mutations and 11 with BRCA2 mutations. Among BRCA2 carriers, tamoxifen was associated with a decrease in risk of breast cancer (risk ratio = 0.32; 95% CI = 0.06 to 1.56); whereas no reduction in risk was observed in the BRCA1 carriers (risk ratio = 1.67; 95% CI 0.32 to 10.7). While these results suggest no benefit of tamoxifen in BRCA1 carriers, the very small number of mutation carriers studied and the attendant lack of statistical significance require that these data be interpreted with caution.

In contrast to the observations from the NSABP-P1 trial, studies of mutation carriers with breast cancer suggest that tamoxifen reduces the risk of contralateral disease. In a case-control study, 209 BRCA1/2 carriers with bilateral breast cancer were compared with 384 BRCA1/2 carriers with unilateral disease (Narod et al. 2000). Tamoxifen use was reported in 21.1% of the women with unilateral breast cancer and by 10.5% of those with bilateral disease. On multivariate analysis, the use of tamoxifen reduced the risk of contralateral breast cancer in BRCA1 carriers (odds ratio = 0.38; 95% CI = 0.19 to 0.74) and BRCA2 carriers (odds ratio = 0.63, 95% CI = 0.20 to 1.50). In a more recent prospective study from the same group, there was a trend toward a reduced risk of contralateral breast cancer in BRCA1 carriers receiving tamoxifen, however, this did not reach statistical significance (hazard ratio = 0.59; 95% CI = 0.26 to 1.33; Metcalfe et al. 2004). Of note, neither of these two studies included
Early age at menarche

Factor

General population

BRCA1 carriers

Early age at menarche
Increase parity
Young age at first term pregnancy
Breast feeding
Oral contraceptive use
Hormone replacement therapy
Oophorectomy
Tamoxifen

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↑*
↓
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– no effect; ↑ increased risk; ↓ decreased risk; * see text.

data on the hormone receptor status of the primary or contralateral breast cancers.

Thus, in conclusion, while the relationship between hormonal factors and breast cancer in BRCA1 carriers is complex, data from clinical studies do suggest that endocrine factors play a role in the development of breast cancer in these women. These factors are summarized in Table 1.

Synthesis and perspectives

We have reviewed evidence that: (i) BRCA1 mutations confer an increased risk for several types of hormone-responsive cancers; (ii) BRCA1 regulates ER-α and AR activity; (iii) hormonal factors may contribute to Brca1-dependent tumorigenesis in mice; and (iv) hormonal factors appear to modify the risk for BRCA1 mutant human breast cancers. These considerations suggest a model of BRCA1 mutant tumorigenesis in which loss of BRCA1’s caretaker function leads to accumulation of mutations within mammary epithelial cells; and these mutant cells are stimulated to proliferate due to loss of the normal regulation of estrogen-stimulated cell proliferation. BRCA1 may also act to promote mammary epithelial cell differentiation, thus reducing the number mammary stem cells that are potential ‘targets’ for cancer development. This model still does not explain the high frequency of ER-α negativity in BRCA1-mutant mouse and human mammary cancers. Here, we postulate that the pathogenesis of these tumors follows a unique pathway in which hormonal stimulation is required early on, followed by loss of hormone receptors as a later event.

While the data we have presented are suggestive, further evidence is required to confirm the contribution of hormonal functions to the etiology of BRCA1-dependent breast cancers. The development of such evidence will require a greater understanding of the molecular pathway(s) of BRCA1-dependent carcinogenesis, particularly the early events in this pathway and the timing and mechanism(s) for the loss of hormone receptor expression. The development of prospective data on the impact of hormone-associated risk factors and hormonal interventions on breast cancer risk in BRCA1 mutation carriers, with a sufficient duration of follow-up, will also assist in determining the physiological relevance of the BRCA1 hormone-regulatory activity. Thus far, the cancer-associated BRCA1 mutations studied in the laboratory confer loss or attenuation of the ability of BRCA1 to mediate DNA repair-related functions as well as hormone-regulatory functions. Based on a greater understanding of the structural basis of the BRCA1: ER-α interaction, it may be possible to engineer a BRCA1 mutation that dissociates these two classes of functions. If so, well-designed animal studies (e.g., ‘knock-in’ of mutant Brca1 genes) could further elucidate the role of hormonal factors in BRCA1-dependent mammary carcinogenesis.

If this hypothesis that a defect in hormonal regulation contributes to BRCA1 is correct, it would suggest that hormonal prevention of BRCA1 mutant breast cancers, whether by oophorectomy, tamoxifen, or other agents, will be most effective if initiated at an early age. Indeed a study of prophylactic oophorectomy in BRCA1 carriers suggests that this procedure is more efficacious in preventing breast cancer if performed at a younger age (Rebbeck 2000). It remains to be settled whether tamoxifen is an effective chemoprevention agent in BRCA1 mutation carriers. We cited a new study showing that tamoxifen stimulates the development of mammary hyperplasias and carriers in Brca1-deficient transgenic mice (Jones et al. 2005). However, the relevance of this study to BRCA1 mutant breast cancers in humans is unclear, since the mice had two mutant BRCA1 alleles, whereas human carriers retain one wild-type allele. Mice heterozygous for the Brca1 exon 11 deletion have not been reported to develop cancer.

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