# Regulation of progesterone receptor signaling by BRCA1 in mammary cancer

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**Inherited mutations of the BRCA1 gene (chromosome 17q21), a tumor suppressor, lead to an increased risk of breast cancer, ovarian cancer, and several other hormone-responsive tumor types. Over the last ten years, BRCA1 has been found to play major roles in DNA damage signaling, repair, and cell cycle checkpoints. In addition, unfolding evidence suggests that BRCA1 functions as a co-regulator for steroid hormone receptors and modulates steroid hormone action. In this paper, we will briefly review this evidence and present a model to address the role of the progesterone and estrogen receptors in BRCA1 mutant mammary carcinogenesis. Finally, we will consider some of the clinical implications of this model.**

Received October 27th, 2005; Accepted February 9th, 2006; Published April 28th, 2006 | **Abbreviations:** **BRCA1:** breast cancer susceptibility gene-1; **ER-α:** estrogen receptor-α; **HRT:** hormone replacement therapy; **MEC:** mammary epithelial cell; **MMTV-Luc:** mouse mammary tumor virus promoter-luciferase reporter; **p300:** E1A-binding protein: 300-kDa; **PR:** progesterone receptor; **PR-A and PR-B:** progesterone receptor isoforms A and B. 

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## Introduction

Mutations of the breast cancer susceptibility gene 1 (BRCA1) are linked to familial breast and ovarian cancer [Miki et al., 1994]. BRCA1 mutation carriers also experience a significantly increased risk for other hormone-responsive tumor types, including uterine, cervical, and prostate cancers [Thompson and Easton, 2002]. Accumulating evidence suggests that BRCA1 functions as a “caretaker” to maintain genomic integrity [Rosen et al., 2003]. However, this function does not explain the predilection of BRCA1 carriers to develop hormone-dependent cancers. We will discuss evidence that physiologic interactions between BRCA1 and steroid hormone receptors [progesterone receptor (PR) and estrogen receptor (ER-α)] contribute to the tissue-specific pattern of tumorigenesis in BRCA1 carriers.

## The PR in mammary cancer

Progesterone physiologically regulates growth in the breast and uterus. The PR, a transcriptional target of ER-α, plays a key role in mammary growth and development, especially during pregnancy. Its role in breast cancer is not as well-established as for ER-α, but available data indicate that PR signaling can stimulate breast cancer development [Conneely et al., 2003; Lange et al., 1999; Schairer, 2002]. Progesterone can exert a biphasic effect on the mammary epithelium, where growth stimulation is followed by inhibition, depending upon the context [Musgrove et al., 1991]. It has been proposed that progesterone primes mammary epithelial cells to respond to other growth regulatory signals [Lange et al., 1999]. Studies in PR-/- mice have uncovered roles for PR in mammary ductal branching and lobulo-alveolar differentiation during pregnancy. A role in cancer is implied by the finding that PR-/- mice are resistant to carcinogen-induced mammary tumorigenesis [Conneely et al., 2003]. In the human menstrual cycle, breast epithelial cell proliferation peaks during the luteal phase, when circulating progesterone levels are maximal, consistent with progesterone stimulation of proliferation in the adult breast [Lange et al., 1999].

Epidemiologic studies have revealed a small but significant increase in breast cancer risk associated with menopausal hormone replacement therapy (HRT) using combined estrogen-progestin treatment, relative to estrogen alone [Schairer, 2002]. In contrast, combined HRT reduces the incidence of endometrial cancer, a tissue where progesterone has anti-proliferative effects. Combined HRT is also associated with higher mammographic density, a marker of breast cancer risk [McTiernan et al., 2005].

## Hormonal factors in BRCA1 mutant breast cancers

While most sporadic breast cancers (60-70%) are hormone receptor positive, most BRCA1 mutant cancers are ER-α and PR negative [Lakhani et al., 2002]. Nevertheless, several lines of evidence suggest an important role for steroid hormones and their receptors in the genesis of BRCA1 mutant cancers. Prophylactic bilateral oophorectomy confers a substantial reduction (about 50%) in breast cancer risk in BRCA1 mutation carriers [Narod, 2001; Rebbeck et al., 2002]; and bilateral oophorectomy reduced the incidence of mammary cancer in mice with a mammary-targeted deletion of full-length Brca1 [Bachelier et al., 2005].

In contrast to sporadic cancers, where early pregnancy has a risk-reducing effect, pregnancy increases the risk of breast cancer or accelerates cancer development in BRCA1 carriers [Narod, 2001]. Early pregnancy is associated with high circulating levels of estrogen and progesterone, suggesting that steroid hormones may confer increased breast cancer risk in BRCA1 carriers. Evidence supporting a hormonal etiology of BRCA1

mutant breast cancers is reviewed elsewhere [Rosen et al., 2005].

A role for Brca1 in mammary development is suggested by the finding that its expression in mice is increased in proliferating cellular compartments that are also undergoing differentiation, including the mammary epithelium during puberty and pregnancy [Lane et al., 1995; Marquis et al., 1995].

Consistent with these findings, endogenous BRCA1 expression is increased during mammary epithelial cell differentiation *in vitro*; and the differentiation process is enhanced by exogenous BRCA1 [Kubista et al., 2002; Rajan et al., 1996]. Furthermore, BRCA1 over-expression can inhibit estrogen and progesterone-stimulated mammary cancer cell proliferation *in vitro* [Ma et al., 2006; Xu et al., 2005], raising the possibility that BRCA1 may coordinate the balance of proliferation versus differentiation in mammary tissue. However, it should be noted that BRCA1-deleted animal models and women show normal mammary differentiation.

## BRCA1 and PR signaling

Previous studies indicate that BRCA1 physically interacts with ER-α and inhibits its transcriptional activity [Fan et al., 2001; Fan et al., 2002; Fan et al., 1999; Kawai et al., 2002; Ma et al., 2005; Xu et al., 2005]; and inactivation of BRCA1 (by mutation or knockdown) confers activation of ER-α in the absence of ligand [Jones et al., 2005; Zheng et al., 2001]. We have extended the scope of these findings by showing that BRCA1 inhibits PR signaling in breast carcinoma cells [Ma et al., 2006]. Thus, we found that in transient transfection assays using a progesterone responsive reporter (MMTV-Luc), exogenous wild-type BRCA1 [but not a cancer-associated mutant (T300G)] inhibited the activity of the PR in progesterone-responsive human breast cancer cells (MCF-7 and T47D). In cells lacking endogenous PR, BRCA1 inhibited the activity of both isoforms of PR (PR-A and PR-B) alone and in combination. Knockdown of endogenous BRCA1 resulted in a four-fold increase in progesterone-stimulated PR activity. Unlike ER-α, BRCA1 knockdown did not confer ligand-independent activation of PR.

## The BRCA1 inhibition of PR activity is due to a physical interaction between the BRCA1 and PR proteins

This interaction differs from the BRCA1: ER-α interaction in several respects: 1) ER-α contacts the N-terminus of BRCA1, whereas PR can contact both N- and C-terminal sites on BRCA1; 2) BRCA1 binds to the AF-2 (activation function 2) domain of ER-α but to a different region on PR; and 3) the nuclear receptor coactivator p300 rescues the BRCA1 repression of ER-α but not PR [Ma et al., 2006]. Over-expression of BRCA1 inhibited the expression of various progesterone-responsive genes just as it blocked expression of most estrogen-inducible genes [Xu et al., 2005]; and just as BRCA1 inhibited estradiol (E2) stimulated cell proliferation in MCF-7 cells [Xu et al., 2005], it also inhibited progesterone-stimulated proliferation of T47D cells [Ma et al., 2006].

We tested the effects of exogenous hormones in mice with a mammary-targeted deletion of the full-length Brca1 isoform (Brca1Co/CoMMTV-Cre). In adult mice with intact ovaries, exposure to a sustained release progesterone pellet for four weeks caused a significant increase in mammary gland volume and tertiary branching in Brca1Co/Co mice but had little effect in wild-type non-transgenic mice [Ma et al., 2006]. In ovariectomized mice, the combination of E2 plus progesterone caused an exaggerated proliferative response in Brca1Co/Co mice, compared to wild-type mice; and a four-week exposure to E2 alone caused a significant increase in mammary epithelial cell (MEC) density in Brca1Co/Co but not wild-type mice. These findings suggest that a Brca1 deficiency abrogates the homeostatic mechanisms that limit the proliferative response to E2 or progesterone alone and enhances the response to the combination of E2 plus progesterone.

In the setting of a heterozygous p53 mutation, Brca1Co/CoMMTV-Cre mice develop invasive mammary cancers [Xu et al., 1999]. Although these tumors are usually ER-α negative (as are most mouse mammary cancers), a recent study suggests the development of these tumors is hormone-responsive. Thus, implantation of a slow-release Tamoxifen pellet significantly enhanced development of mammary cancers in Brca1Co/CoMMTV-Cre/p53+/- mice [Jones et al., 2005]. Tamoxifen also stimulated development of mammary hyperplasias, in which expression of ER-α was lost. Consistent with these findings, knockdown of BRCA1 in cultured MCF-7 cells caused a significant increase in Tamoxifen-stimulated ER-α activity, suggesting that BRCA1 may regulate the relative agonist versus antagonist activity of Tamoxifen.

## Model for hormone receptor role in development of BRCA1 mutant breast cancers

Based on these considerations, we propose a model for BRCA1 mutant breast cancer formation (Figure 1). Here, ER/PR-positive MECs deficient for BRCA1 are hyper-sensitive to endogenous E2 and progesterone and secrete growth factors that stimulate proliferation of nearby ER/PR-negative MECs. Paracrine mechanisms of this type occur during normal mammary development [Conneely et al., 2003]. Continual hormonal stimulation results in ER/PR-negative hyperplasias. In the setting of genomic instability due to BRCA1 deficiency, these lesions eventually become autonomous and progress to invasive cancer. During the evolution of these tumors, the p53 gene becomes mutated, since BRCA1 mutant breast cancers exhibit a very high incidence (approximately 80%) of p53 mutations, suggesting this is an obligate event [Phillips et al., 1999]. A value of this hypothesis is that it is testable in available animal models. Alternatively, BRCA1 mutant hyperplasias and cancers may be derived from MECs that were originally

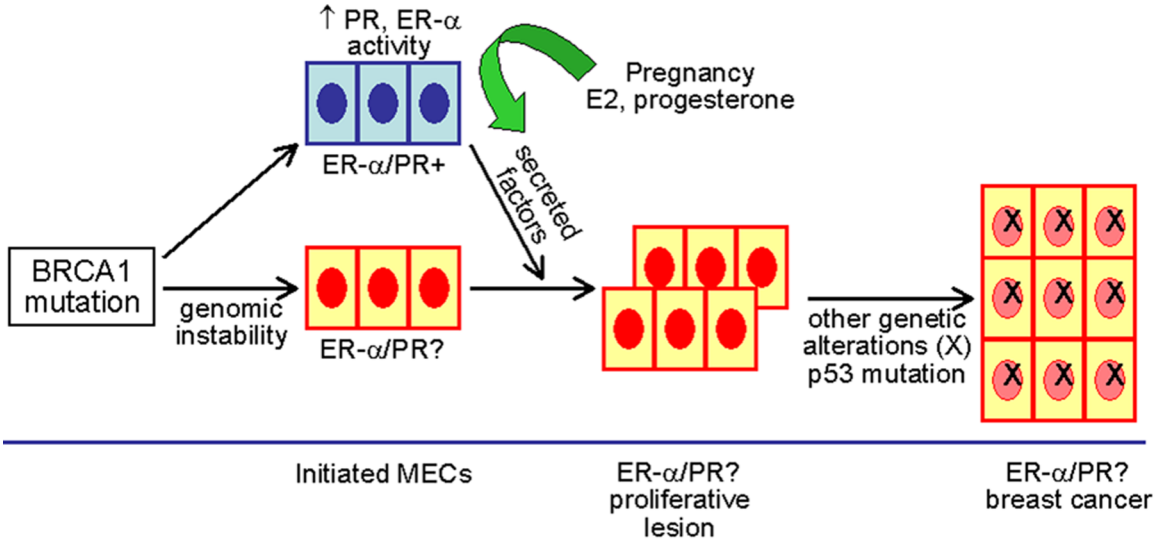


**Figure 1. Hypothetical model showing role of hormone receptor signaling in the development of BRCA1 mutant breast cancer.** In the absence of functional BRCA1, ER-α/PR positive mammary epithelial cells (MECs) are hypersensitive to E2 (estradiol) and progesterone and stimulate proliferation of hormone receptor negative cells (eg., by secretion of growth factors or by other mechanisms). This results in ER-α/PR negative proliferative lesions (hyperplasias or dysplasias), which ultimately progress to invasive cancer in the setting of genomic instability due to loss of BRCA1 function.

ER-α/PR-positive and converted to ER-α/PR negativity as a consequence of hormone-stimulated cell proliferation, chromosomal instability due to loss of BRCA1 function [Tirkkonen et al., 1997], or epigenetic silencing.

A recent study supports the hypothesis of a paracrine interaction involving hormone receptor positive and negative cells in BRCA1 mutant cancer development. Thus, PR expression was more common in normal mammary epithelial cells adjacent to an invasive breast cancer in BRCA1 mutant cancers than in sporadic cancers [King et al., 2004]. The wild-type BRCA1 allele was retained in the microdissected normal mammary epithelium cells. One possibility is that progesterone stimulates the PR+ surrounding non-cancer cells to secrete growth factors that stimulate proliferation of PR negative cells that become the cancer, but this remains to be proved.

## Clinical implications

This model, if validated, provides a rationale for using an anti-progestin, alone or in combination with an anti-estrogen, for breast cancer prophylaxis in BRCA1 mutation carriers. The National Surgical Adjuvant Breast and Bowel Project P1 breast cancer prevention trial, which showed a 50% overall risk reduction in women treated with Tamoxifen, did not show a significant benefit for Tamoxifen in BRCA1 carriers [King et al., 2001]. It should be noted that the number of BRCA1 carriers in the NSABP-P1 trial was small; and the efficacy of Tamoxifen in preventing BRCA1 mutant breast cancers or as adjuvant treatment for BRCA1 mutant cancers is not settled [Rosen et al., 2005]. A subset of sporadic breast cancers (? 30-40%) show reduced or absent BRCA1 expression due to promoter methylation, loss of one BRCA1 allele, or other causes [Esteller et al., 2000; Staff et al., 2003; Taylor et al., 1998; Wilson et al., 1999]. Loss of BRCA1 inhibition of PR activity could contribute to development of a subset of sporadic breast cancers, providing a rationale for the use of an anti-progestin to treat premenopausal breast cancers that are PR-positive and under-express BRCA1.

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