Estrogens, BRCA1, and Breast Cancer

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Abstract

Findings obtained in in vitro assays and animal studies indicate that estrogens might influence the activity of the tumor suppressor gene BRCA1, and BRCA1 in turn may suppress the activity of the estrogen receptor. This review will discuss the possibility that interactions between estrogens and BRCA1 partly explain why elevated circulating estrogen levels appear to increase breast cancer risk among postmenopausal women but not among young women. A hypothesis is proposed that estrogens have a dual role in affecting breast cancer risk. In young women whose breasts have not yet accumulated critical mutations required for cancer initiation and promotion, activation of BRCA1 by estrogens helps to maintain genetic stability and induce differentiation, and therefore estrogens do not increase breast cancer risk. Breasts of older women, in contrast, are likely to contain transformed cells whose growth is stimulated by estrogens. Although BRCA1 is also probably activated by estrogens in older women, its function may have been impaired, for example, due to increased methylation associated with aging. Estrogen exposure in women who carry germ-line mutations in BRCA1 may always increase breast cancer risk because estrogens would be able to cause DNA damage and increase genetic instability without being opposed by BRCA1-induced repair activity. This might lead to an increase in the number of overall mutations, including those that initiate breast cancer. In addition to increasing genetic instability, reduced BRCA1 activity may also be linked to changes in the mammary gland morphology that predispose individuals to breast cancer. For example, a persistent presence of lobules type 1, which are the least differentiated lobular structures in the human breast, is seen in the BRCA1 mutation carriers. The aim of this review is to discuss the role of premenopausal estrogens in breast cancer and to initiate more research that would lead to novel means of reducing breast cancer risk, particularly among BRCA1 mutation carriers.

Introduction

The role of estrogens in affecting breast cancer risk during premenopausal years has remained largely unknown. Several factors related to reproduction appear to predispose women to breast cancer. For example, women with early onset of menarche (menstruation begins at <12 years) or late menopause (menopause occurs after 55 years) have an increased risk of developing breast cancer (1). These findings suggest that the longer the exposure to ovarian estrogens, the higher the risk. This view is supported by the fact that surgically induced menopause before age 45 years and the resulting removal of ovarian estrogens markedly reduce breast cancer risk (2–5). Furthermore, the partial ERβ agonist tamoxifen, which blocks the actions of estrogens in the breast, effectively prevents primary and recurring breast tumor development (6). However, it is not clear that there is a correlation between high estrogen exposure and high breast cancer risk during the years when women have functional ovaries. In fact, an increase in breast cancer risk may be seen after a modest reduction in circulating estrogens, such as that produced by unilateral ovariectomy (4, 5), oral contraceptive (7, 8) or contraceptive depot use [both of which inhibit ovulation and ovarian estrogen production (9, 10)], or low body weight and low fat intake (11–14). In contrast, an increase in exposure to circulating estrogens during premenopausal years caused by several pregnancies (15), short menstrual cycle length (16, 17), high BMI (18), or a high fat intake (11) may reduce the risk of developing breast cancer. High BMI or fat intake are indirect indicators of increased estrogenicity: a considerable amount of estrogen production occurs in adipose tissue, which is a site for conversion of adrenal androgens to estrogens, particularly in prepubertal girls and postmenopausal women.

A hypothesis is proposed here that estrogens might play a dual role in affecting breast cancer risk. On one hand, there is evidence to indicate that estrogens might serve as preinitiators, initiators, and promoters of breast cancer. We generally associate estrogens with promotion of the growth of existing malignancies in the breast (Fig. 1). However, these hormones and their metabolic products are also shown to induce direct and indirect free radical-mediated DNA damage, genetic instability, and mutations in cells in culture and in vivo (19), suggesting a role for estrogens in cancer initiation. Furthermore, estrogens may serve as preinitiators. For example, elevated fetal estrogen levels can permanently alter the morphology of the mammary gland (20) and cause a persistent presence of epithelial structures (TEBs) that are known to be sites of malignant growth (21). Data obtained in animal models and indirect evidence in humans indicate that high in utero estrogenicity increases breast cancer risk (20, 22, 23).

In contrast to these adverse effects of estrogens on the breast, in certain circumstances, such as during pregnancy that occurs before age 20 years (1, 15, 24) and during the prepubertal period and childhood (11, 14, 25), estrogens actually reduce breast cancer risk. The reduced risk could be achieved through estrogen-induced activation of certain tumor suppressor genes, including BRCA1 (26–28) and p53 (29) that are critical in DNA damage repair and in maintaining genetic stability, thus reducing the likelihood that breast cancer will be initiated. The interaction between estrogens and tumor suppressors might be important during the early reproductive years, when the breast does not yet contain any malignancies. Once breast cancer initiation has taken place, estrogens might promote the growth of transformed cells, leading to the development of detectable breast cancer. Because estrogens increase BRCA1 expression in human breast cancer cells in vitro (27, 28), they are also likely to do so in women whose breast contains malignant cells. However, given that breast cancer initiation has already occurred, the function of one or more tumor suppressors may be impaired at this point (failure in tumor suppressor gene function is believed to contribute to cancer initiation). In women carrying a mutated BRCA1 gene, estrogens may always induce genetic instability because the mutated BRCA1 is
unable to correct genetic alterations. Evidence is presented below to support this hypothesis.

Why Would Estrogen Exposure during Reproductive Years not Increase Breast Cancer Risk?

In addition to the reproductive system and the breast, estrogens are required for the development and function of many other tissues. Estrogens are of critical importance in bone development and the maintenance of bone density (30, 31) and a healthy cardiovascular system (32, 33). Estrogens are also required for neuronal growth and differentiation, and these hormones are linked to cognitive functions (34) and mood (35, 36). Because estrogens possess several essential functions, it would be surprising if a complementary system did not exist in parallel with estrogens to protect tissues like the breast from the adverse effects of estrogens.

Estrogens exhibit both beneficial and harmful effects on the breast. The breast undergoes periods of varying sensitivity to the adverse effects of estrogens (37). These hormones are needed during normal breast development, particularly during puberty and pregnancy (38). For example, although pregnancy markedly increases circulating estrogen levels, pregnancy before age 20 years reduces breast cancer risk (24). In contrast, a similar pregnancy-induced increase in estrogen levels after age 30 years increases breast cancer risk (1). One explanation for the differential effects of estrogens during pregnancy is that this gene is tightly linked to the regulation of cellular proliferation (42). This conclusion is supported by observations that BRCA1 mRNA levels exhibit a cell cycle-dependent pattern: expression is low in cells arrested in $G_0$ or early $G_1$ and highest at the $G_1$-S-phase transition (42–44). BRCA1 protein also undergoes hypophosphorylation during late $G_1$ and S phases, indicating that the protein is then being activated. However, BRCA1 expression is not limited to cell proliferation. Rajan et al. (42) have shown that Brca1 mRNA levels are high in postconfluent HC11 mammary epithelial cells during differentiation and when treated with insulin and glucocorticoids. Because proliferation rates under these conditions are low, and differentiation is high, Brca1 also appears to be involved in the process of differentiation of the breast.

The work by Gowen et al. (45) suggests that BRCA1 plays a key role in repairing oxidative DNA damage. This probably indicates that in rapidly proliferating tissues, BRCA1 may help to maintain the integrity of the genetic material. Furthermore, BRCA1 interacts with RAD51, a protein that has been implicated in DNA recombination and repair (46). The fact that BRCA1 has also been identified as a p53-interacting protein (47) lends further support to the idea that BRCA1 may be involved in repairing DNA damage. BRCA1 has been shown to act as a transcriptional coactivator and increase the p53-dependent transcription from P21 and BAX promoters (47). DNA-damaging agents trigger a transient induction of p53, and this gene has been strongly implicated in DNA damage repair.

Besides p53, several other proteins that interact with BRCA1 have been identified, including c-myc, BAP-1 (48), and retinoblastoma susceptibility gene RB1 (49). The c-myc oncogene is closely linked to breast carcinogenesis, and it is one of the early response genes activated in $G_1$ phase, resulting in the activation of a number of other genes with important roles in cell cycling. It has been suggested that BRCA1 down-regulates c-myc activity (48). BAP-1 is a novel protein found on the basis of its interaction with BRCA1 (50). BAP-1 might enhance BRCA1-mediated growth inhibition, at least in human breast.
cancer cells (50). Inherited mutations in one of the RB1 alleles result in the development of retinoblastoma and/or osteosarcoma and increase susceptibility to other cancers. It was recently shown that the product of the RB1 gene, Rb, regulates the expression of both the murine Brca1 and human BRCA1 genes (49). BRCA1 also transactivates the expression of p21, the major cyclin-dependent kinase inhibitor involved in the inhibition of cell cycle progression and induction of apoptosis, in a p53-independent manner (51).

In summary, BRCA1 has been implicated to have a primary role in DNA damage response by processing signals that arise after damage (48). This role results from cross-talks with other critical elements of signal transduction pathways and causes cell cycle arrest, DNA repair, and perhaps apoptosis.

**Estrogens and BRCA1**

The fact that Brca1 expression is induced during puberty and pregnancy, when estrogen levels are dramatically increased, suggests that estrogens might stimulate the expression of this gene. This suggestion is supported by a finding showing that E2, together with progesterone, increases the level of Brca1 expression in the mammary glands of ovariec-tomized mice (26). Studies in ER-positive MCF-7 and BT20 human breast cancer cells indicate that depletion of estrogens significantly reduces BRCA1 mRNA expression, and the expression is increased again by treatment with E2 (27, 28). It should be noted that no estrogen-responsive element has been identified within the promoter of the BRCA1 gene, and the increase in BRCA1 mRNA expression by estrogens probably occurs via an estrogen-initiated increase in overall RNA synthesis (52).

It is essential to determine how and why estrogens stimulate BRCA1 expression. BRCA1 mRNA expression and ER mRNA expression are closely linked to each other, suggesting a functional relationship between the two genes (53). Furthermore, methylation of the BRCA1 promoter appears to be strongly correlated with a lack of ER or progesterone receptor expression (54). In accordance with these observations, BRCA1 was recently shown to have an ability to regulate the cellular response to estrogens (55). In *in vitro* studies conducted using human breast cancer cells, BRCA1 protein inhibited ER-α-mediated transcriptional pathways related to cell proliferation. This finding suggests that in addition to maintaining genomic stability during periods of rapid cellular division and multiplication, BRCA1 may also suppress signaling initiated by estrogen-induced activation of ER-α. Thus, during puberty and pregnancy, when estrogens and BRCA1 expression are both significantly increased, the function of BRCA1 may be to protect the breast from estrogen-induced genetic instability by inhibiting ER-mediated pathways, inducing differentiation, and repairing genetic damage. BRCA1 might also be particularly important in controlling cellular proliferation. A loss of BRCA1 function leads to increased proliferation of malignant cells in cell culture (56, 57), and stable transfection of wild-type BRCA1 into these cells inhibits their growth (58). However, activation of BRCA1 seen during puberty and pregnancy does not seem to block proliferation occurring in the breast at these times.

**BRCA1 and Breast Cancer**

Germ-line mutations only occur in one BRCA1 allele because homozygous deletion of BRCA1 is lethal in utero. However, germ-line BRCA1 mutation carriers who develop breast cancer often exhibit loss of heterozygosity of the wild-type BRCA1 locus (59). Thus, both BRCA1 alleles appear to be lost in those breast cancer cases where BRCA1 is the precipitating genetic lesion. It is possible that a loss of one allele, through a germ-line mutation, may alter the function of other genes, leading to a dramatic genomic instability. This instability then creates an environment in which the loss of function of wild-type BRCA1 is highly likely. It is not inconceivable that loss of wild-type BRCA1 is not a causative factor in the development of inherited breast cancer but rather a side effect.

Somewhat surprisingly, somatic mutations in BRCA1 are extremely rare in sporadic breast cancer (40, 60). Instead, in sporadic breast cancers, the level of normal BRCA1 protein is often reduced either through loss of heterozygosity of one BRCA1 allele or by other means (56, 61). Down-regulation of the normal BRCA1 may be caused, for example, by alternative splicing (62), aberrant methylation (63– 65), or defects in subcellular localization of the BRCA1 protein (66). Failure of transcriptional regulation by ER may also be responsible for reduced BRCA1 mRNA levels in sporadic breast cancer (67).

The level of BRCA1 expression in sporadic breast cancer is related to the degree of invasiveness of the tumor. Compared with normal breast tissue, BRCA1 expression is lowest in invasive cancer (56) and is intermittently reduced in *in situ* carcinomas (68). However, the latter finding has not been confirmed in all studies, and there are reports indicating higher BRCA1 mRNA levels in ductal carcinoma *in situ* than in normal mammary epithelium (56). The decreased BRCA1 expression might be a causal event, reflecting tumor progression, or a secondary effect caused by changes in upstream regulatory pathways controlling BRCA1 expression (69). In either case, reduced BRCA1 expression rather than loss of function of both alleles is linked to sporadic breast cancer.

Taken together, BRCA1 mutations appear to cause breast cancer only when present in the germ line, although the reasons for this are unknown. Furthermore, in germ-line mutation carriers, both alleles are lost at the time the tumor is detected, whereas in sporadic breast cancer, BRCA1 expression is reduced but not completely lost. This suggests that a loss of function of wild-type BRCA1 in germ-line mutation carriers has to occur, whereas this is not the case in sporadic breast cancer. It is possible that BRCA1 may function differently in embryonic versus adult cells (69). During embryogenesis, BRCA1 is of critical importance for cell proliferation; thus, homozygous germ-line lesions result in an early cell proliferation defect that kills the embryos. Consequently, cellular events leading to breast cancer might be different in BRCA1 mutation carriers versus women who develop sporadic breast cancer but exhibit reduced BRCA1 expression as adults. In the mutation carriers, the presence of only one functional BRCA1 allele *in utero* may create an environment of increased genetic instability, increasing the probability that mutations in other critical genes will occur. This argument is supported by the fact that p53, another tumor suppressor gene, which is called “the guardian of the genome,” is more frequently inactivated in BRCA1 mutation-associated tumors than in sporadic breast cancer (70, 71). Up to 90% of BRCA1 mutation-associated tumors harbor a p53 mutation and/or p53 protein accumulation [which occurs due to either a mutation in the p53 gene or alterations in p53 upstream signaling pathways (71)]. These events might also lead to a loss of wild-type BRCA1, which may or may not be essential for tumor initiation.

In sporadic breast cancer, normal BRCA1 function might be reduced due to various environmental factors. The environmental exposures that may alter BRCA1 expression levels in women who develop sporadic breast cancer include PAHs or changes in circulating estrogen levels. PAHs are widely present in our environment, and they reduce BRCA1 mRNA expression in human breast cancer cells (72). We propose that BRCA1 levels might be reduced by an exposure to a low estrogenic environment. Changes in estrogen exposure levels can be caused by differences in the amount of adipose tissue or differences in the use of contraceptive drugs, hormone replacement therapy, or exposure to environmental estrogens. It is possible that an
interindividual variability in estrogen levels in women contributes to the lowering and increasing of BRCA1 expression.

There is some indirect evidence that estrogen-induced activation of BRCA1 may be important in protecting the breast. For example, the phytoestrogen genistein (73), which is a major active component in soy products and may reduce the risk of developing premenopausal breast cancer (74), increases the expression of BRCA1 in human breast cancer cells in culture (75). Because genistein exhibits weak estrogenic activities, and estrogens also up-regulate BRCA1, the results suggest that estrogenic compounds may reduce breast cancer risk by activating normal BRCA1. Another observation in support of the estrogen/Brca1 link is that high estrogenicity before the onset of puberty in animal models reduces breast cancer (76–78), and Brca1 is up-regulated at puberty (26). Increased estrogenicity before the initiation of ovariogen production might activate BRCA1 earlier than normal, which could further help to maintain genomic stability at puberty. The surge of ovarian estrogens at puberty is likely to increase estrogen-induced DNA damage and repair mechanisms. This speculation is based primarily on results obtained in animal models. However, high prepubertal estrogenicity might also protect the human breast. It has been noted that in humans, indicators of high estrogenicity during childhood and early premenopausal years are linked to inhibition rather than initiation of breast cancer (14, 25). Thus, estrogenic exposures at these times, perhaps through estrogen-induced activation of BRCA1, may be involved in reducing the probability that normal cells would later turn to the malignant pathway.

It has been suggested that the rarity of BRCA1 mutations in sporadic breast cancer is due to the greater likelihood of BRCA1 inactivation by nonmutational mechanisms than by mutation. One nonmutational mechanism of BRCA1 inactivation that has been observed in sporadic breast cancer is methylation (64). Hypermethylation of CpG-rich areas located within the promoter of genes may be a common mechanism of silencing tumor suppressor genes. Hypermethylation has been shown to increase with age (79), and if this occurs in the BRCA1 gene, it could help to explain why estrogens decrease breast cancer risk in older women. Theoretically, exposing older women to estrogens results in tumor promotion that methylated BRCA1 cannot prevent. Besides aging, it is not known what induces hypermethylation in women with sporadic breast cancer. One of the pathways could be through an exposure to various environmental agents that promote hypermethylation of important cancer-related genes (80), possibly including BRCA1. The possibility also exists that low circulating E2 levels might contribute to the induction of hypermethylation (81).

**BRCA1 Mutation Carriers and Estrogens**

If one of the BRCA1 alleles is lost due to a mutation, as is the case in familial breast cancer, estrogens might be more likely to cause genomic instability than if both alleles were functioning normally. This would mean that estrogen exposure, particularly during puberty and young adulthood, increases the penetrance of breast cancer in germ-line BRCA1 mutation carriers. Although approximately 70% of women who carry a germ-line BRCA1 mutation will develop breast cancer by age 70 years (82), the remaining 30% do not. It is not known whether the age at onset of puberty or menopause, circulating estrogen levels, body weight, diet, exercise, alcohol intake, or other factors that affect estrogen levels alter breast cancer risk among germ-line BRCA1 mutation carriers. Oral contraceptives, when used before first pregnancy, may increase breast cancer risk in BRCA1 carriers (83). In contrast, smoking appears to reduce breast cancer risk in these women (84). Oral contraceptive use reduces the exposure to ovarian estrogens, and exposure to the synthetic estrogen is lower than that to estrogen originating from the ovaries. Smokers are reported to have lower circulating estrogen levels than nonsmokers, although the association has not been confirmed in all studies (85–87). Thus, based on these observations, it cannot be determined whether high levels of estrogens increase breast cancer risk in women with BRCA1 mutations.

However, there are four important observations that suggest that estrogens may indeed increase the penetrance of breast cancer in BRCA1 mutation carriers. First, men heterozygous for BRCA1 mutations do not exhibit an increased incidence of breast cancers (88), indicating that low estrogen and/or high androgen levels might be protective. Second, bilateral prophylactic ovariectomy is associated with a significantly reduced breast cancer risk in women who carry a BRCA1 mutation (89). Third, women possessing germ-line mutations in BRCA1 are particularly susceptible to breast cancer as a result of pregnancy (90, 91). Pregnancy increases circulating estrogen levels by approximately 10-fold. Fourth, women with a strong family history of breast cancer (approximately 50% of these women are BRCA1 mutation carriers, and most of the others carry a mutation in some other tumor suppressor gene) exhibit a 4-fold increase in breast cancer risk if they had a high BMI at the age of 12 years (92). As indicated above, a high BMI during childhood clearly reduces sporadic breast cancer risk (14, 25). The last two findings strongly suggest that a mutated BRCA1 cannot protect the breast from the cancer-initiating/promoting effects of estrogens.

Women who do not carry germ-line BRCA1 mutations but have lost the function of the normal BRCA1 gene by other means should also exhibit an estrogen-induced increase in breast cancer risk. Such a loss may be more likely to have occurred in older versus younger women. Relatively consistent evidence shows that elevated estrogen levels during postmenopausal years increase breast cancer risk. Postmenopausal women who have high circulating estrogen levels (93, 94), are obese (95), or are exposed to hormone replacement therapy (96) exhibit an increase in breast cancer risk, although not all studies support these findings. The probability of genetic mutations is believed to increase with age, but no evidence exists thus far to indicate that the BRCA1 gene is mutated in older women (or in young women, for that matter) who develop sporadic breast cancer. However, older women may have acquired mutations in genes in which BRCA1 acts as a coactivator, such as p53, and this could potentially lead to a reduction in BRCA1 activity as well. As discussed above in connection to the differential effect of pregnancy on breast cancer risk in young and older women, breasts of older women are more likely to contain preneoplastic and neoplastic cells than those of young women. Based on what we currently know about cancer initiation (loss of function of tumor suppressor genes and overexpression of oncoproteins allow normal cells to turn to a malignant pathway), the function of one or more tumor suppressor genes, possibly including BRCA1, in women with preneoplastic lesions is more likely to have been lost in older women than in young women. Methylation of BRCA1 is also among the potential mechanisms that could inactivate this gene in older women (64, 79). Thus, although estrogens stimulate BRCA1 in older women, BRCA1 is not able to repair and maintain genomic stability because it has lost its function (or function is impaired) in the process that has allowed preneoplastic lesions to occur in the first place.

**Estrogens, BRCA1, and Mammary Gland Morphology**

It is generally believed that mutations in tumor suppressor genes and oncogenes are required for breast cancer initiation to occur. However, alterations in normal communications between stroma and parenchyma, perhaps reflecting or occurring in parallel with epige-
netic changes, might be essential in tumor formation. It has been argued that the structure of the breast tissue has to be critically altered for malignant transformation to progress, even in the presence of multiple chromosomal mutations (97). We have been studying changes in the mammary epithelial tree in mice and rats exposed to estrogenic compounds during the in utero period. In utero estrogenic exposures that increase breast cancer risk in animal models increase the number of TEBs in the offspring’s mammary gland and prevent their differentiation (20, 98). TEBs contain a rapidly proliferating population of epithelial cells and drive ductal growth (21). In mice and rats, the presence of TEBs is highest around puberty, and the TEBs subsequently differentiate to lobulo-alveolar units, becoming virtually nonexistent in the adult gland (21). Animal data indicate that TEBs are the primary targets for carcinogen-induced malignant growth (21), and the corresponding structure in the human breast, terminal ductal lobule unit, may also be the site most susceptible to the development of human breast cancer (99). Interestingly, in animals, the mammary structure exhibiting the highest level of Brca1 mRNA is the TEB in virgin animals and the alveoli during pregnancy (26).

It was noted recently that breasts of women who are germ-line BRCA1 mutation carriers exhibit a high number of the least differentiated lobules type 1, regardless of whether they are parous or not (100). Normal (noncarrier) women show a long-lasting reduction in the number of lobules type 1 and an increase in the number of well-differentiated lobules type 3 after pregnancy (101). It is possible that the persistent presence of lobules type 1 in the BRCA1 mutation carriers results from an interaction between high in utero estrogen exposure and one nonfunctional BRCA1. Another possibility is that one mutated BRCA1 gene is sufficient to prevent normal differentiation of the human breast that occurs during pregnancy.

High estrogenicity during fetal life may contribute to high breast cancer incidence among BRCA1 mutation carriers. Although estrogen levels are significantly higher in pregnant women than in nonpregnant women (102), there is still a 4–6-fold variability in these levels among women who are undergoing apparently normal pregnancies. Thus, some pregnant women (and their fetuses) are exposed to significantly higher levels of estrogens during pregnancy than other pregnant women. It has been hypothesized that the highest range of fetal estrogen exposure levels increases later breast cancer risk compared with the lowest range of fetal estrogen exposure levels (22, 103). This hypothesis is supported by indirect epidemiological evidence showing that high birth weight, which is linked to a high fetal estrogenic environment (104), increases breast cancer risk (105, 106). Dizygotic twins also are exposed to an increased fetal estrogenic environment and exhibit increased breast cancer risk as adults (107, 108). However, a recent study comparing pregnancy estrogen levels between Asian and Caucasian women suggests that high pregnancy E2 levels may not increase breast cancer risk if birth weight is not simultaneously increased (109).

In accordance with the changes seen in the mammary gland morphology in animals exposed to high in utero estrogenicity, high placental weight in humans, which indicates high fetal estrogen exposure, is associated with high density mammographic parenchymal patterns (110). High density mammographic patterns, in turn, are associated with increased breast cancer risk (111). It remains to be determined whether or not the high number of lobules type 1 in the breasts of BRCA1 mutation carriers reflects high fetal estrogen levels, and whether or not they contribute to increasing breast cancer risk in these women also remains to be determined.

Another observation suggesting a link between high in utero estrogenicity and breast cancer in BRCA1 mutation carriers is that in utero exposure to a high estrogenic environment reduces total ER content (including both the classical ER-α and novel ER-β subtypes) in the normal mammary gland and in breast tumors (112–114). Breast cancers in BRCA1 carriers are often ER-α negative (115, 116). This could indicate that germ-line BRCA1 mutation carriers who develop breast cancer are those who also were exposed to the highest range of estrogen levels during fetal life, which then down-regulated ER expression in the breast. However, it can also be argued that continuous adult exposure to high estrogen levels both down-regulates breast ER levels and increases breast cancer risk in women who are BRCA1 mutation carriers. Whether or not low ER levels in the breast are causally related to the development of breast cancer in the mutation carriers is not known.

**Brca1 and Mammary Tumorigenesis in Animal Models**

In animal models, loss of one Brca1 allele is not sufficient to promote cancer. For example, although homozygous deletions of Brca1 in knockout mouse models are lethal early in embryonic development (117–119), mice carrying heterozygous deletions of Brca1 are phenotypically normal and do not exhibit an increased predisposition to tumorigenesis (120). However, when the remaining Brca1 gene is inactivated in mature heterozygous brca1 knockout mice, mice will develop breast cancer (121).

There is no evidence that reduced Brca1 expression is related to carcinogen-induced rodent breast cancer models or to models in which mammary tumors are seen in mice exhibiting activated neu or activated ras oncogenes. This appears to contradict the human data showing reduced Brca1 expression in sporadic breast cancer versus normal tissue. Brca1 mRNA expression levels are similar in mammary tumors induced by carcinogens 7,12-dimethylbenz(a)anthracene or methyl-nitrosourea or by activation of neu or ras oncogenes and in nonmalignant cells (122). The key to understanding the apparent species difference in Brca1 expression in tumors and nonmalignant tissues might lie in the factors that cause breast cancer in women versus rats. In women, the underlying genetic and molecular events that initiate breast cancer have remained largely unknown, whereas in animal models, the causal factor is apparent (for example, carcinogen exposure or overexpression/knockout of a specific gene). Environmental exposures and changes in hormonal status might play a major role in human breast cancer, although the details are far from being clear. It is possible that these hormonal/environmental factors lead to reduced Brca1 expression. For example, a recent study indicates that the PAH benzo(a)pyrene reduces Brca1 mRNA levels in MCF-7 human breast cancer cells (72). This, in turn, might cause increased genetic instability and cell proliferation, mutations in a gatekeeper gene, and, finally, breast cancer. In most rodent models, it is carcinogens and oncogenes that induce mammary tumors, not hormonal/environmental factors, although they clearly affect the promotion and progression stage of rodent mammary tumorigenesis and may serve as preinitiators (20).

**Tumor Suppressor Gene p53**

Germ-line and somatic mutations in the p53 tumor suppressor gene predispose carriers to a wide variety of cancers, including breast cancer (123). Mutation of p53 is the most common somatic alteration in sporadic breast cancer, with an estimated frequency of 12–46% in invasive breast cancers (124). p53 has an ability to recognize and bind to damaged DNA, repair it, and induce both cell cycle arrest and apoptosis (125). p53 has thus been categorized as both a caretaker and gatekeeper tumor suppressor gene (126).

Like BRCA1, expression of p53 mRNA may be modulated by estrogens. T47D human breast cancer cells exhibit a reduction in p53 expression when grown in medium depleted of endogenous steroids, and subsequent E2 administration increases p53 expression (29). Fur-
BRCA1 in women who develop sporadic breast cancer.

In addition to the fact that both p53 and BRCA1 seem to be induced by estrogens and play a role in DNA repair, their relationship is shown to be more than merely general. Several lines of evidence indicate that p53 is associated with breast cancer in BRCA1 mutation carriers. First, mutations in p53 occur at a high frequency in tumors of BRCA1 mutation carriers (70). Second, BRCA1 enhances p53-mediated transcription (51), as evident from the observation that transfection of cells with mutated BRCA1 inhibits p53-mediated transcription of effector genes (47). Furthermore, BRCA1 stimulates many p53-responsive genes, although it can inhibit p53-mediated transcription of effector genes (47). Further-from the observation that transfection of cells with mutated BRCA1 also stimulates expression of these genes independent of p53 (47).

Second, BRCA1 enhances p53-mediated transcription (51), as evident from the observation that transfection of cells with mutated BRCA1 inhibits p53-mediated transcription of effector genes (47). Furthermore, BRCA1 enhances p53-mediated transcription (51), as evident from the observation that transfection of cells with mutated BRCA1 inhibits p53-mediated transcription of effector genes (47). A mutation in p53 may also be associated with down-regulation of BRCA1 in women who develop sporadic breast cancer.

It has been suggested that because E2 promotes human breast cancer cell proliferation, the induction of p53 may indicate that in vitro E2 stimulates p53 to regulate proliferation (129). We propose that this also applies in vivo in the human breast. Thus, premenopausal women exposed to elevated estrogen levels may also exhibit an increase in p53 expression. If p53 is normal, it guards the genome against somatic mutations that might initiate cancer; if it is mutated or silenced by other means, it is unable to prevent increased genetic instability induced by estrogens, and breast cancer risk is increased.

Other Tumor Suppressor Genes

Besides BRCA1 and p53, other tumor suppressor genes have been identified, including BRCA2. In theory, they may protect the breast from the adverse effects of estrogens. For example, the BRCA2 gene on chromosome 13q is another tumor suppressor gene linked to heritable breast cancer (130, 131). There are striking similarities in the expression patterns and functions of Brca1 and Brca2 (26, 69). Brca2 is expressed in the same tissues and cell types as Brca1 (42, 132). Furthermore, BRCA2 expression is also induced by estrogens (28) and is high during puberty and pregnancy (42, 132). It is plausible that many other tumor suppressor genes will be identified in the breast that are either stimulated or inhibited by estrogens or independent of these hormones. Estrogens at different time points during development, as well as the level of estrogenicity originating from ovaries versus that of non-gonadal estrogens, are likely to affect breast cancer risk in a manner that is determined by a response to the total network of signaling pathways of estrogens.

Conclusions

There is a considerable amount of confusion among scientists and lay people as to whether the risk of breast cancer can be reduced by altering lifestyle, including dietary modifications and exercise patterns. A low-fat diet is known to reduce serum estrogen levels, but low body weight does not reduce premenopausal breast cancer risk (11–14, 25). A high fiber content reduces circulating estrogen levels by increasing fecal excretion of the hormone (133) but does not consistently reduce breast cancer risk (134). Similarly, exercise reduces estrogen levels (135) without necessarily reducing the risk of developing breast cancer (136). The consistent findings indicating that an early onset of puberty increases breast cancer risk are believed to support the idea that early estrogen exposure increases breast cancer risk; however, it may merely reflect an exposure to an elevated in utero estrogenic environment because this environment both accelerates puberty onset and increases breast cancer risk (20). Therefore, it is of critical importance to clarify the link between estrogens, particularly changes in estrogenicity induced by lifestyle factors, and human breast cancer.

We propose that estrogens have a dual role in affecting breast cancer risk by interacting with tumor suppressor genes on one hand and by stimulating cell proliferation, as summarized in Fig. 2, on the other hand. Kinzler and Vogelstein (126) describe BRCA1 as a “caretaker” tumor suppressor gene. A caretaker’s role is to maintain
the integrity of the genome. Thus, high estrogen levels may increase normal BRCA1 expression in an attempt to ensure genomic stability in the face of a potential estrogen-induced increase in genomic damage. Mutated BRCA1 in inherited breast cancer or down-regulated BRCA1 in sporadic breast cancer is unable to repair genomic damage induced by high levels of estrogens, increasing the likelihood that other mutations will occur and that a normal cell will ultimately become transformed. This interaction between estrogens and BRCA1 probably explains, at least in part, why BRCA1 mutation carriers exhibit a significantly increased risk of breast cancer and a moderately increased cancer risk in other estrogen-regulated sites (ovaries, prostate, and possibly the colon), but not in non-estrogen-regulated tissues. One way to test the hypothesis that an interaction between estrogens and BRCA1 determines whether estrogens increase or reduce breast cancer risk is to determine BRCA1 expression levels in relation to BMI, fat intake, or circulating estrogen levels in women to find out whether these factors are associated with tumor suppressor activity. Another way to test the hypothesis is to determine whether women who carry a germ-line BRCA1 mutation show an increase in breast cancer penetrance when exposed to the highest levels of estrogens in utero (i.e., women who had a high birth weight) or during puberty (i.e., women who consumed a high-fat diet or had a high BMI). If this turns out to be true, perhaps penetrance of breast cancer in BRCA1 mutation carriers can be reduced by dietary modifications that reduce pregnancy estrogen levels and body weight throughout life.

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