Estrogen Carcinogenesis in Breast Cancer

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In this article, we review recent findings related to estrogen exposure and the risk of breast cancer, the mechanisms that may be involved, and the clinical implications of these findings. The weight of evidence indicates that exposure to estrogen is an important determinant of the risk of breast cancer. The mechanisms of carcinogenesis in the breast caused by estrogen include the metabolism of estrogen to genotoxic, mutagenic metabolites and the stimulation of tissue growth. Together, these processes cause initiation, promotion, and progression of carcinogenesis. Insight into the mechanisms of the causation of cancer by estrogen will identify determinants of susceptibility to breast cancer and new targets for prevention and therapeutic intervention.

HORMONAL RISK FACTORS FOR THE DEVELOPMENT OF BREAST CANCER

An association between the risk of breast cancer and persistently elevated blood levels of estrogen has been found consistently in many studies. Several endocrine-associated risk factors are regularly associated with an increased relative risk of breast cancer in postmenopausal women.1-3 One of these factors is obesity, which is probably related to an increased production of estrogen by aromatase activity in breast adipose tissue.4 Another factor is an elevated blood level of endogenous estrogen (relative risk, 2.00 to 2.58).5 An increased relative risk is also associated with higher-than-normal blood levels of androstenedione and testosterone, androgens that can be directly converted by aromatase to the estrogens estrone and estradiol, respectively. Elevated urinary levels of estrogens and androgens are also associated with an increased risk of breast cancer in postmenopausal women.6 The level of serum progesterone has not been associated with a risk of breast cancer in postmenopausal women,7 whereas in premenopausal women, blood levels of progesterone appear to be inversely associated with the risk of breast cancer.8,9 All this evidence supports the hypothesis that cumulative, excessive exposure to endogenous estrogen across a woman’s life span contributes to and may be a causal factor in breast cancer.

Observational epidemiologic studies10-12 and a randomized clinical trial13-15 have investigated the long-term effects of replacement therapy — with estrogen alone (estrogen-replacement therapy) or with estrogen plus progestin (hormone-replacement therapy) — on various health outcomes, including breast cancer. In the United States, the main replacement preparations contain conjugated estrone plus various conjugated equine estrogens16 alone or combined with medroxyprogesterone acetate. A meta-analysis of data from 51 observational studies involving more than 160,000 women determined that for users of hormone- or estrogen-replacement therapy for 5 years or longer (average duration of use, 11 years), the relative risk was 1.35 (95 percent confi-
A recent observational study of more than 54,000 French women showed a similar statistically significant increase in risk (relative risk, 1.4; 95 percent confidence interval, 1.2 to 1.7) for women using hormone-replacement therapy but not estrogen-replacement therapy. In the Million Women observational study of unselected women in the United Kingdom recruited between 1996 and 2001, the relative risk of breast cancer was significantly elevated by the use of hormone- or estrogen-replacement therapy and increased with the duration of use. However, the risk was higher than those reported in other studies. Within a year after the cessation of use, the relative risk returned to that of patients who had never received replacement therapy, again suggesting that the effects of such therapy are reversible. Although a strength of this study was its large size, it had important weaknesses, including reliance on a single questionnaire and an enrollment bias that may have inflated the relative risks. Nevertheless, the results from all these observational studies, when taken together, suggest that ongoing or recent hormone-replacement therapy is associated with a small but significant increase in the risk of breast cancer (albeit reversible) and that estrogen replacement may have similar effects.

This question was tested in the Women’s Health Initiative study, a prospective trial in which postmenopausal women were randomly assigned to receive placebo or hormone-replacement therapy (if they had a uterus) or either placebo or estrogen-replacement therapy (if they had undergone hysterectomy). The study was terminated for both groups after a median follow-up of about five years because of the increased incidence of breast cancer, stroke, coronary heart disease, and pulmonary embolism in the group receiving hormone-replacement therapy and increased incidence of stroke and pulmonary embolism in the group receiving estrogen-replacement therapy, as compared with placebo. In the group receiving hormone-replacement therapy, the risk of breast cancer, expressed as the hazard ratio, was significantly increased. These results confirm the increased risk of hormone-replacement therapy observed in the Collaborative Group meta-analysis, the recent European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study, and the Million Women study, although the extent of the increase as represented by the hazard ratio cannot be compared directly with the increase represented by the relative risk. However, unlike the Collaborative Group meta-analysis and the Million Women study, the Women’s Health Initiative study found no increased risk of breast cancer in the group receiving estrogen-replacement therapy, whose members had undergone hysterectomy. Finally, the use of oral contraceptives is also associated with a small but significant increase in the risk of breast cancer during the time of use.

Why hormone-replacement therapy and estrogen-replacement therapy may have different influences on the risk of breast cancer is not clear. It is not unexpected that the combination of estrogen and progestin increases the risk of breast cancer, since progestins tend to increase cell proliferation in breast tissue and have a variety of other effects on the breast. Medroxyprogesterone acetate alone increases the incidence of mammary tumors in mice and dogs, but it was not associated with a significant increase in the relative risk of breast cancer and endogenous progestin levels were not associated with an increased risk of breast cancer in women who used the drug as a contraceptive. Collectively, the results of epidemiologic studies of endogenous steroid hormone levels and the use of exogenous estrogen support the hypothesis that estrogen contributes to and may have a causal role in breast cancer, although the contribution to breast cancer of progestin associated with hormone-replacement therapy requires additional investigation.
in various tissues, including the kidneys, liver, uterus, and mammary glands. Figure 1 outlines two different but complementary pathways that together are likely to contribute to the carcinogenicity of estrogen.

**ESTROGEN METABOLISM**

Figure 2 shows estrogen metabolism through the catechol pathway. Phase I metabolism in humans, hamsters, mice, and rats involves several cytochrome P-450 enzymes that catalyze the oxidative metabolism of estrone and estradiol predominantly to a 2-hydroxycatechol estrogen. The estrogen 3,4-quinone can form unstable adducts with adenine and guanine in DNA, leading to depurination and mutation in vitro and in vivo. Reduction of estrogen quinones back to hydroquinones and catechols provides an opportunity for redox cycling to produce reactive oxygen species and probably accounts for the oxidative damage to lipids and DNA that is associated with estrogen treatment.

Phase II detoxication pathways — including sulfation, methylation, and reaction with glutathione — are active in breast tissue for protection against
damage caused by reactive metabolites of endoge-
ous and exogenous chemicals. Figure 2 shows
where catechol estrogen methylation catalyzed by
catechol O-methyltransferase and estrogen semi-
quinone and quinone reaction with glutathione can
occur. In addition to preventing metabolism of
catechol estrogen to quinones, the 2-methoxy cate-
chol may be a protective metabolite.47-52 Of inter-
est, 4-hydroxyequilenin, a reactive catechol metab-
olite of equilenin, an equine estrogen present in
hormone-replacement-therapy preparations, can
inhibit detoxication enzymes such as glutathione
S-transferase P1-1 and catechol O-methyltransferase.
53,54 Furthermore, recombinant catechol O-
methyltransferase with low activity was found to
be more sensitive than the native enzyme to inhibi-
tion by 4-hydroxyequilenin.55 This suggests that
reactive equine estrogen metabolites contribute
to breast cancer through inhibition of protective
phase II enzymes and raises the possibility that
women who are homozygous for the polymorphic
variant of catechol O-methyltransferase with a low
activity could be at further increased risk when
using hormone-replacement-therapy preparations
containing equine estrogen.

Table 1 presents a selected summary of the bio-
logic effects of treatment of cultured cells and ex-
perimental animals with estradiol or its metabo-
lites. These observations support the hypothesis
that oxidative metabolites of estrogen have geno-
toxic, mutagenic, transforming, and carcinogenic
potential and thus could initiate or cause the pro-
gression of the carcinogenic process in humans.
However, to date, no studies have definitively dem-
onstrated that estrogen metabolites contribute to
human breast cancer, although two lines of evidence
support this possibility. First, the hypothesis that
potentially genotoxic estrogen metabolites contrib-
ute to human breast cancer depends on their for-
mation and presence in breast tissue. In post-
menopausal women, estrogen levels in breast tissue
are 10 to 50 times the levels in blood,64 and concen-
trations of estradiol were higher in malignant
tissues than in nonmalignant tissues, a finding that

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**Figure 2. Oxidative Metabolism of Estrogen through the Catechol Pathway.**

Data are from Cavalieri et al.32 E1 denotes estrone, E2 estradiol, COMT catechol O-methyltransferase, P-450 cytochrome
P-450, and GST glutathione S-transferase.
probably reflects aromatase activity in breast tissue. Furthermore, levels of estrogen metabolites and conjugates ranging from 3 to 13 pmol per gram of tissue were detected in human breast tissue, demonstrating that the oxidative pathways shown in Figure 3 are active in human breast tissue. However, larger studies are needed to confirm these findings. It will be necessary to detect estrogen–quinone adenine and guanine adducts and oxidative DNA damage in human breast tissue to provide definitive evidence for estrogen genotoxicity that could contribute to the initiation or progression of breast cancer.

The second line of evidence supporting a role for estrogen metabolites in human breast cancer comes from studies of associations of breast-cancer risk and polymorphisms in genes encoding enzymes involved in estrogen synthesis and metabolism (Table 2). The products of these genes are involved in estrogen synthesis (e.g., cytochrome P-450 17 and cytochrome P-450 19; the latter is also called aromatase), aspects of phase I metabolism that might lead to increased levels of metabolites (e.g., cytochrome P-450 1A1 and cytochrome P-450 1B1), and actions of phase II metabolism that might lead to reduced protective conjugation (e.g., glutathione S-transferase M1 and catechol 0-methyltransferase). Overall, the results of these studies have been inconsistent.

Such mixed results probably reflect the small size of the studies and the low penetrance of mutations in genes in these multigenic pathways. A typical example is represented by the studies on catechol O-methyltransferase. After the initial report of the association of homozygosity for an allele encoding a low-activity form of the enzyme with an increased risk of breast cancer (odds ratio for postmenopausal women, 2.2; 95 percent confidence interval, 0.9 to 5.2; odds ratio for postmenopausal women with a body-mass index [the weight in kilograms divided by the height in meters] ≥24.5, 3.6; 95 percent confidence interval, 1.1 to 12), at least 10 subsequent studies explored this issue. Approximately half the studies detected an association, some in premenopausal women and some in postmenopausal women. A recent study observed combined effects of several reproductive factors and variant alleles for catechol O-methyltransferase and the glutathione S-transferases M1 and T1 with an increased risk of breast cancer. However, because the number of subjects of cases and controls in the combined groups was small, this finding requires independent confirmation. Ritchie et al. explored the association of combinations of 10 single-nucleotide polymorphisms in catechol O-methyltransferase, cytochrome P-450 1A1 and 1B1, and glutathione S-transferases M1 and T1 with the risk of breast cancer and observed that an interaction among the low-activity variant of catechol O-methyltransferase, two variants of

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<th>Table 1. DNA Damage, Mutation, and Cell Transformation Associated with Estrogen and Estrogen Metabolites.</th>
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<td><strong>Effect</strong></td>
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<td>Estrogen 3,4-quinone DNA adducts and depurination</td>
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<td>Oxidative DNA damage</td>
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<td>Neoplastic transformation</td>
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* ACI denotes a cross between August and Copenhagen–Irish strains and SENCAR sensitive to carcinogenesis.
cytochrome P-450 1B1, and a variant of cytochrome P-450 1A1 were significantly associated with an increased risk of breast cancer, although these findings too require independent confirmation. Future efforts to determine whether polymorphisms that alter the expression or activities of genes that encode estrogen-metabolism enzymes influence susceptibility to breast cancer will require an assessment of their association with estrogen metabolite levels in breast tissue or secretions from both case subjects and control subjects.

**ESTROGEN-RECEPTOR SIGNALING**

Figure 3 summarizes the multiple estrogen-receptor signal-transduction pathways, emphasizing effects associated with increased proliferation and inhibition of apoptosis. Table 3 lists selected examples of signaling events mediated by estrogen receptors.

The classic mechanism of direct action of estrogen on nuclear DNA involves the binding of the hormone to nuclear estrogen receptors, which then bind as dimers to estrogen-response elements in the regulatory regions of estrogen-responsive genes and associate with basal transcription factors, co-activators, and corepressors to alter gene expression. Since the initial discovery and characterization of the estrogen receptor α (ERα) in the 1960s, research on mechanisms of estrogen-receptor signaling has revealed its complexity, as represented by the discovery of estrogen receptor β (ERβ) and...
of signaling pathways mediated by estrogen receptors that become associated with mitochondria and the plasma membrane. ERα and ERβ have 96 percent amino-acid identity in their DNA-binding domains, whereas there is only 53 percent homology in their ligand-binding domains; the latter accounts for differences in the responses of the two receptors to various ligands. For example, tamoxifen has been reported to be both an agonist and an antagonist for ERα but only an antagonist for ERβ. Also, ERβ has a greater affinity for various phytoestrogens, such as genistein, than does ERα. The receptors also differ in their activation domains, suggesting that they may recruit different proteins to the transcription complexes, thereby altering the specificity of their genomic transcriptional effects. Furthermore, estrogen receptors interact with coactivator proteins to stimulate the activity of other transcription factors, such as AP-1 (Table 3). Finally, various tyrosine kinase growth-factor receptors can activate estrogen receptors by phosphorylation in the absence of ligand (Table 3).

The presence of specific, high-affinity estrogen binding in nonnuclear subcellular fractions, including plasma membrane and mitochondria, implies that the estrogen receptor could be located at these sites. Indeed, recent studies have definitively shown the presence of ERα, ERβ, or both in mitochondria of various cells and tissues. The mitochondrial genome contains potentially estrogen-responsive sequences, and estrogen has increased mitochondrial DNA-encoded gene transcript levels (Table 3). The mechanisms of mitochondrial estrogen-receptor import are not known, although ERβ contains a putative mitochondrial-targeting peptide domain. Studies are needed to elucidate the mechanisms of estrogen-receptor import, to determine how it functions to increase transcription of mitochondrial DNA, and to determine the role of the process in the response to estrogen.

Like peptide growth factors, estrogens also cause

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<th>Table 2. Functional Effects of Selected Polymorphisms in Selected Estrogen Synthesis and Metabolizing Genes Associated with an Increased Risk of Breast Cancer.</th>
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<td><strong>Enzyme</strong></td>
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<td>CYP 19</td>
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<td>Phase II enzyme</td>
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<td>GSTM1</td>
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<td>COMT</td>
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* Data are from Mitrunen and Hirvonen, Ritchie et al., and Miyoshi and Noguchi. CYP denotes cytochrome P-450, GSTM1 glutathione S-transferase M1, COMT catechol O-methyltransferase, and PCB polychlorinated biphenol.
activation of various protein kinases, such as mitogen-activated protein kinases, and increase levels of second messengers, such as cyclic AMP (cAMP), within minutes (Table 3). These nongenomic, nontranscriptional effects involve a membrane-bound form of ERα, ERβ, or both and facilitate cross-talk between the membrane estrogen-receptor–signaling process and other signal-transduction pathways, such as the epidermal growth factor receptor and insulin-like growth factor 1 receptor–signaling pathways (Fig. 3).

Together, these new insights into multiple genomic and nongenomic estrogen-signaling pathways greatly expand our understanding of the potential cross-talk among various signal-transduction pathways. However, a thorough investigation is required of all the interacting signaling pathways mediated through the estrogen receptor and growth factors, such as epidermal growth factor and insulin-like growth factor 1, that function in human breast tissue in normal and tumor cells. This could facilitate the goal of achieving a comprehensive understanding of the control of proliferation and apoptosis by estrogen and its metabolites and perhaps reveal new targets for combined therapeutic intervention.

**Clinical Implications**

Clinical evidence also supports a role for estrogen in mammmary carcinogenesis. The ability of estrogen withdrawal to suppress breast-cancer growth was...
demonstrated in 1896 by Beatson, who performed oophorectomy for palliation of advanced breast cancer in young women. The seemingly paradoxical later finding that pharmacologic doses of estrogen also inhibit the growth of breast cancer is explained by the recent observation that estrogen can also trigger apoptotic pathways, particularly after a period of estrogen deprivation.

The strongest evidence for the role of estrogen in breast cancer has emerged from the experience with the selective estrogen receptor modulator tamoxifen for the treatment and prevention of breast cancer. Individual trials and a meta-analysis of randomized clinical trials have shown that tamoxifen reduces the risk of recurrence for women of any age with invasive or in situ breast cancer that expresses ER, the progesterone receptor, or both. These studies also showed that tamoxifen reduces the risk of new breast cancer in the contralateral breast, an observation that was the basis for randomized chemoprevention trials comparing tamoxifen or raloxifene with placebo. A meta-analysis of these studies suggests that tamoxifen reduces the risk of breast cancer by 38 percent in healthy women at high risk for breast cancer. The reduction in risk appears to be confined to ER-positive tumors, consistent with a hypothesis that tamoxifen’s primary effects are mediated through estrogen-receptor pathways. Results from recent clinical trials with aromatase inhibitors, agents that suppress estrogen synthesis through peripheral aromatization, in postmenopausal women with ER- or progesterone-receptor-positive breast cancer reinforce the importance of estrogen in breast-cancer growth. Several large, randomized trials have compared aromatase inhibitors with tamoxifen in postmenopausal women with early or advanced steroid-receptor–positive breast cancer. Women who were treated with aromatase inhibitors had superior outcomes and a lower incidence of cancer in the contralateral breast than did women who received tamoxifen. Aromatase inhibitors are also now under study for chemoprevention in healthy postmenopausal women at high risk for breast cancer.

These approaches have mainly targeted the classic estrogen-receptor pathway by reducing the amount of ligand or receptor or interfering with receptor–ligand interactions. However, the importance of the nonclassic pathways described above is increasingly evident. Preclinical models suggest that resistance of some steroid-receptor–positive breast cancers to agents like tamoxifen may be abrogated by the use of receptor tyrosine kinase inhibitors and clinical studies are in progress to test the role of combination therapy targeting classic and nonclassic signaling.

It is likely that improved understanding of the molecular biology of breast cancer will rapidly advance the field. Expression-profiling studies suggest that breast cancers can be molecularly subclassified. For example, one molecularly defined subtype, termed luminal A, demonstrates an estrogen-receptor–derived gene-expression profile, whereas a second subtype, termed basal, lacks estrogen-receptor expression; these findings imply that estrogen-related factors are integral to the development and treatment of the luminal A subtype but play a lesser role in the emergence of the basal subtype. It may also be possible to discern subtle molecular differences in the effects of selective estrogen-receptor modulators. Results of expression profiling of a breast-cancer cell line (MCF-7) that was treated with various modulators suggest that tamoxifen and raloxifene have similar effects, whereas the action of fulvestrant (an estrogen-receptor down-regulator) was distinct. Thus, an improved understanding of estrogen-signaling pathways may enhance our understanding of the development of breast cancer and facilitate tailored interventions in appropriately selected patients.

**CONCLUSIONS**

Studies of breast cancer have consistently found an increased risk associated with elevated blood levels of endogenous estrogen, clinical indicators of persistently elevated blood estrogen levels, and exposure to exogenous estrogen plus progestin through hormone-replacement therapy and the use of oral contraceptives. In experimental animals, estrogen treatment leads to the development of mammary tumors. Together, these observations support the hypothesis that estrogen is a mammary-gland carcinogen.

The mechanisms through which estrogens contribute to each phase of the carcinogenic process (initiation, promotion, and progression) are complex. The evidence suggests the participation of genotoxic estrogen metabolites and estrogen-recep-
tor-mediated genomic and nongenomic signaling that affect cell proliferation and apoptosis in mammary tissue. The extent to which these two pathways contribute to estrogen-mediated carcinogenesis and the ways by which genetic polymorphisms and environmental factors modify the effects of these pathways require further exploration. Even so, knowledge of the central role of estrogen in breast cancer has already led to the development of new preventive and therapeutic interventions that block receptor function or drastically reduce the levels of endogenous estrogen through the inhibition of its synthesis. The development of additional strategies on the basis of the inhibition of estrogen metabolism, inactivation of the reactive quinones, and specific inhibition of membrane estrogen-receptor–activated second-messenger pathways will probably lead to the availability of additional effective intervention approaches.

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**RESEARCH PERSPECTIVES**

1. The use of aromatase inhibitors could be a potential treatment strategy.
2. Genistein and other isoflavones may have a role in preventing breast cancer.
3. Better understanding of the metabolic pathways involving xenobiotics will enhance safety assessment.
4. Estrogen modulation of carcinogenesis in mammary tissue is a promising area for study.

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