Estrogen and Cancer

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**Abstract**

Estrogen exhibits a broad spectrum of physiological functions ranging from regulation of the menstrual cycle and reproduction to modulation of bone density, brain function, and cholesterol mobilization. Despite the beneficial actions of endogenous estrogen, sustained exposure to exogenous estrogen is a well-established risk factor for various cancers. We summarize our current understanding of the molecular mechanisms of estrogen signaling in normal and cancer cells and discuss the major challenges to existing antiestrogen therapies.

**Keywords**
estrogen receptor, breast cancer, tamoxifen, endocrine resistance
ESTROGEN METABOLISM IN NORMAL AND CANCER CELLS

Estrogen sex steroid hormones exhibit a broad spectrum of physiological functions ranging from regulation of the menstrual cycle and reproduction to modulation of bone density, brain function, and cholesterol mobilization (1–3). Despite the normal and beneficial physiological actions of endogenous estrogen in women, abnormally high estrogen levels are associated with the increased incidence of certain types of cancer, especially those of the breast and endometrium. In 1991, the Women’s Health Initiative (WHI) research program initiated a 15-year study aimed at evaluating the beneficial effects of postmenopausal hormone replacement therapy (HRT) on heart diseases, bone fractures, and cancers; 161,808 generally healthy women of ages 50–79 were enrolled in this study. The WHI was terminated prematurely in 2002 due to the increased incidence of breast cancer, stroke, and cardiovascular complications in women treated with estrogen alone or with a combination of estrogen and progestogen (4). Shortly afterward, the US National Toxicology Program made the unprecedented decision to classify estrogens as carcinogens. The International Agency for Research on Cancer (IARC) also listed both estrogen and estrogen-progestogen-combined postmenopausal therapies as known human carcinogens. In this review, we focus on the mechanisms of estrogen action in carcinogenesis and discuss current antiestrogen strategies in cancer treatment. Due to space constraints, we apologize in advance to the many outstanding researchers whose work we cannot cite.

The predominant intracellular estrogen is 17β-estradiol (E2). Other types of estrogen include estrone (E1) and estriol (E3) (Figure 1a). In premenopausal women, E1 and E2 are secreted primarily by the ovaries during the menstrual cycle, with minor levels derived from adipose tissue and the adrenal glands. The placenta also produces E3 during pregnancy. In the ovaries, granulosa cells synthesize estrogen from androgen (5). Ovarian production of estrogen is regulated by the hypothalamic-pituitary-ovarian (HPO) axis and begins by anterior pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to the hypothalamic peptide gonadotropin-releasing hormone (GnRH). Acting in concert, LH stimulates androgen production, whereas FSH upregulates aromatase, which catalyzes the rate-limiting and final step of estrogen biosynthesis: the aromatization of androgen to estrogen. During ovulation, E2 production rises dramatically by eight- to tenfold. High levels of estrogen in turn act via negative feedback to dampen estrogen production to inhibit the release of GnRH, LH, and FSH (6).

The primary mediator of estrogen biosynthesis in postmenopausal women is aromatase, which is found in adipose tissue as well as in the ovaries, placenta, bone, skin, and brain (7, 8). After menopause, ovarian estrogen biosynthesis is minimal, and circulating estrogen is derived principally from peripheral aromatization of adrenal androgen. As such, for obese postmenopausal women, adipose tissue becomes the main source of estrogen biosynthesis; this biosynthetic route is far less significant for nonobese postmenopausal women (9). Although aromatase is critical to maintaining normal estrogen levels in both premenopausal and postmenopausal women, these groups respond differently to aromatase inhibitors (AIs), which are used as antiestrogen therapy to treat breast cancer. In premenopausal women, the HPO axis is functional, with estrogen levels changing dramatically throughout the menstrual cycle. As a result, the effects of AIs are quickly overridden when estrogen biosynthesis is driven by the next cycle of GnRH, LH, and FSH secretion. Thus, AIs are much more efficacious in postmenopausal women, whose HPO axis is no longer active. In men, the testes also produce significant amounts of aromatase, allowing for androgen aromatization and estrogen synthesis (10).

Numerous studies have demonstrated the association of estrogen with the development and/or progression of various types of cancer, including cancers of the breast, endometrium, ovary,
prostate, lung, and colon (3, 11). Estrogen is a classical etiological factor for breast cancer and endometrial cancer, and most of our current knowledge of estrogen action comes from clinical and laboratory studies of these two types of cancer.

MOLECULAR MECHANISMS OF ESTROGEN ACTION

Estrogen mediates its biological effects in target tissues primarily by binding to specific intracellular receptors, estrogen receptor (ER)\(\alpha\) and ER\(\beta\). Since the initial reports of ER more than 50 years ago (12), tremendous efforts have been made to decipher the complex molecular mechanisms of estrogen–ER action. In the classical model of estrogen–ER function, the complex acts as a ligand-activated transcription factor to regulate the expression of multiple target genes. An alternative view is that estrogen exerts rapid, stimulatory effects on intracellular signal transduction pathways. This nongenomic pathway begins outside the nucleus and is independent of gene transcription (13–15).

ER\(\alpha\) and ER\(\beta\) are encoded by \(ESR1\) and \(ESR2\), respectively; each gene is located on a different chromosome. ER\(\alpha\) and ER\(\beta\) are widely expressed in many tissues, such as the uterus, ovary, mammary gland, prostate, lung, and brain. The expression levels and subtypes of ER are primary
factors that determine tissue-specific estrogen responsiveness (16, 17). Both ERα and ERβ are members of the nuclear hormone receptor superfamily of ligand-regulated transcription factors that contain modular structures and discrete functional domains (Figure 1b). ERα and ERβ are highly homologous (~96%) in their DNA-binding domains (DBDs) and possess moderate (53%) sequence identity in their ligand-binding domains (LBDs). The major functional difference between ERα and ERβ appears to be determined by the hormone-independent transcriptional activation function (AF-1) domains in their respective N termini (18). The pro-oncogenic effect of estrogen is mediated primarily by ERα activation of target genes that promote cell proliferation or decrease apoptosis.

The binding of estrogen to ERs induces conformational changes in protein structure that allow receptor dimerization and interaction with coactivators. In some cases, the activated estrogen-ER complex directly binds to estrogen response elements (EREs) in gene promoters, leading to the transcriptional activation of target genes. Alternatively, the estrogen-ER complex indirectly activates gene transcription through protein-protein interactions with other transcription factors such as activator protein 1 (AP1), specificity protein 1 (SP1), nuclear factor-κB (NF-κB), cAMP response element–binding protein (CREB), runt-related transcription factor 1 (RUNX1), p53, and signal transduction and activator of transcription 5 (STAT5) (2, 19–22). Interaction with other transcription factors allows the estrogen-ER complex to regulate target genes whose promoters do not harbor a bona fide ERE.

The relative abundance of ERα versus ERβ in a given cell type appears important for mediating tissue-specific estrogen responsiveness (16). ERβ was discovered in 1996, almost 40 years after the initial characterization of ERα (18). Genetic and pharmacological lesions directed against either ERα or ERβ show that these two estrogen nuclear receptors exert opposing effects on cell proliferation and apoptosis (23–26). Another variant of ERα, the truncated ERα-36, appears to mediate membrane-initiative effects of estrogen signaling, and as does ERβ, ERα-36 interferes with ERα activity, as well as with antiestrogen treatment of breast cancer (27). A recent review summarized various ER subtypes and their roles in cancer biology and therapy (16).

To gain full transcriptional regulatory activity, the estrogen-ER complex needs to recruit diverse transcriptional coregulators, many of which have various enzymatic activities to modify chromatin structures or to interact with the general transcription apparatus. More than 300 proteins have been shown to interact with one or more members of the nuclear receptor superfamily, and many of these proteins also interact with ER (13, 28). The p160 family proteins (or SRDs), including three distinct but related homologous members—SRC-1, SRC-2 (GRIP1), and SRC-3 (AIB1)—are the best-characterized coregulators of ER (29). SRCs facilitate a transcriptionally permissive environment through direct and/or indirect recruitment of other coactivators that have chromatin-remodeling and histone modification activities. In addition, SRC proteins possess intrinsic but weak histone acetyltransferase (HAT) activity and mediate transcriptional activation by directly acetylating conserved lysine residues on histones and possibly other coregulators (30, 31). Although all three SRCs directly interact with ER, they recruit different sets of secondary cofactors to further fine-tune the transcriptional regulatory activity of the ER-nucleated apparatus (32). The assembly and disassembly of the ER complex are extremely dynamic, and variation in gene expression among tissues under different conditions can greatly affect the final composition of the ER complex.

ERs, as well as their coregulators, are subjected to a variety of posttranslational modifications (PTMs), which further influence the stability, subcellular localization, transactivity, and hormone sensitivity of the ER-nucleated transcriptional apparatus. Approximately 22 sites throughout ERα are subjected to various modifications, including phosphorylation, methylation, acetylation, sumoylation, and ubiquitination. For example, ER phosphorylation by several kinases,
such as mitogen-activated protein kinase (MAPK) and protein kinase A (PKA), enhances ER transcriptional activity (33, 34); ER acetylation by the HAT p300 regulates ER transactivity and hormone sensitivity (35); and ER ubiquitination leads to ER degradation and thus reduces ER bioavailability (36). A recent comprehensive review summarizes the different types of ER PTMs and their biological functions (37). Besides ER, various coregulators are also subjected to PTMs. PTMs of coregulators change their protein conformation and greatly affect the subunit composition, potency, and target selectivity of the final ER complex. For example, coactivator-associated arginine methyltransferase 1 (CARM1), a well-established coactivator of ERα and a type I protein arginine methyltransferase, catalyzes asymmetric dimethylation of arginine residues (38). Substrates of CARM1 include histone H3 (39), the ER-interacting HATs CBP and p300 (40), the p160 family member AIB1 (41), and the C-terminal domain of RNA polymerase II (42). Accordingly, CARM1 regulates ERα-mediated gene transcription in multiple ways from changing local chromatin structures to affecting the general transcription apparatus and specific cofactors. PKA phosphorylates CARM1. Whereas unphosphorylated CARM1 binds to ERα in the classic ligand-dependent manner, phosphorylated CARM1 binds to the unliganded LBD of ERα and regulates the expression of different sets of genes (43).

Many genes involved in multiple intracellular pathways are directly regulated by the large estrogen-ER transcription apparatus. Over the past several years, several groups have sought to identify all ER target genes in the genome to fully understand the biological functions of estrogen.

Prior to genomic approaches, a hypothesis-driven, candidate gene approach yielded some well-characterized ER target genes, such as trefoil factor 1 (TFF-1, also known as pS2) and cathepsin D, that have been used to delineate the molecular mechanisms of estrogen signaling (44). The rapid advancement of array-based technology and next-generation sequencing has greatly facilitated the identification of novel ER target genes. Data obtained by these technologies have transformed our perception of the way that ER functions (Figure 2). In the conventional view, ligand-bound ERs function at the promoter regions of target genes. Genome-wide mapping of ER-binding sequences of human chromosomes 21 and 22 by using ChIP-tilling arrays indicates that the majority of the ER-binding sites are distant (as far as several hundreds of kilobases away) from the transcription start sites of regulatory genes and that these distal ER-binding domains often function as transcriptional enhancers. Moreover, many of these binding sites lack a consensus ERE but are also bound by Forkhead protein FoxA1, which is obligate for a genuine ER-chromatin interaction (45, 46). ChIA-PET (chromatin interaction analysis using paired-end tag sequencing) technology, which couples chromosome conformation capture with high-throughput sequencing, allows for the global identification of three-dimensional chromatin loops formed in cells upon estrogen induction. Treating hormone-deprived MCF-7 breast cancer cells with estrogen for a short period yields 689 ER-associated chromatin interaction complexes, which are made up of duplexes and more complex interactions. By combining the ChIA-PET data with gene expression profiles, Fullwood et al. (47) discovered that a significant percentage of these clusters are transcription hot spots induced by estrogen. The actual anchor points in these clusters can be separated by hundreds of kilobases or even by megabases in linear two-dimensional chromatin, suggesting that long-range chromatin interactions between DNA elements play key roles in estrogen-induced transcriptional regulation (47).

Although ERα and ERβ have similar affinities for E2 and bind similar DNA response elements, each receptor exhibits a unique genomic signature (45, 46, 48). Genomic profiling and ChIP-Seq studies find that gene networks involved in cell proliferation are activated differently by these two estrogen receptors, implying that a balance of ERα and ERβ signaling ultimately determines how estrogen acts.

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Figure 2
Molecular mechanisms of estrogen-induced gene transcriptional regulation. E2 diffuses into the cell and binds to ER (ERα/ERβ). Liganded receptors enter the nucleus and form homo- or heterodimeric complexes that bind to estrogen-response elements (EREs) on the target gene promoters. Liganded receptors can also act as monomers and bind to target gene promoters through interaction with other transcription factors such as Sp1 and Ap1. Promoter-associated ERs initiate gene transcription through interaction with coregulators and the basal transcriptional machinery. Researchers recently found that ER can bind to enhancer elements far distally from target genes regulated by ER. In some cases, the presence of the pioneer factor FoxA1 is necessary for ER to bind to its DNA response element.

ESTROGEN SIGNALING IN CARCINOGENESIS
Mechanistic studies have established a solid foundation for our understanding of estrogen signaling in carcinogenesis. Genetic changes associated with the oncogenic effects of estrogen match well with the classic genetic markers of human cancers. Indeed, the activated estrogen-ER complex regulates various genes that play key roles in cell proliferation and cell cycle progression. The mitogenic effects of estrogen are particularly clear at the G1-to-S transition, during which the key effectors of estrogen action are c-Myc and cyclin D1 (49, 50). One of the earliest responses to estrogen is increased c-Myc expression, which occurs within 15 min of estrogen stimulation. Estrogen also rapidly induces cyclin D1 expression, and antiestrogen agents have a converse
acute, inhibitory effect. Inhibition of c-Myc or cyclin D1 activity abrogates estrogen-stimulated breast cancer cell proliferation, whereas induction of c-Myc or cyclin D1 can mimic the effects of estrogen and reinitiate cell cycle progression in antiestrogen-arrested cells (51). Although the genes encoding c-Myc and cyclin D1 have been well established as estrogen-induced genes for more than 20 years, the underlying molecular mechanisms of estrogen action on these two genes are still not fully understood. In the case of the c-Myc gene, it was originally proposed that an atypical estrogen-responsive region in the c-Myc proximal promoter is the cis element where activated ER binds and functions. However, recent studies indicate that cooperation between ER and AP1 at a distal enhancer element upstream of the transcriptional start site of c-Myc is responsible for estrogen-induced c-Myc expression (52). Unlike ERα, ERβ inhibits estrogen-induced cell proliferation. Forced expression of ERβ in ERα-positive breast cancer T47D cells inhibits the proliferative responses to E2, possibly by suppressing the expression of cyclin E but not that of cyclin D1 (53).

Estrogen inhibits apoptosis by upregulating antiapoptotic Bcl-2 and Bcl-XL expression in breast cancer cells (54). The estrogen–ERα complex interacts with proteins such as c-Src through nongenomic action and activates the MAPK and PI3K/Akt pathways, which are classically linked to cell survival (19). Paradoxically, estrogen induces apoptosis under certain circumstances. Before the introduction of tamoxifen, high-dose estrogen was used to induce tumor regression of hormone-dependent breast cancer in postmenopausal women. This regimen is of clinical interest, given that long-term treatment of breast cancer with antiestrogen tamoxifen often leads to drug resistance and that sustained tamoxifen exposure may sensitize breast cancer cells to high-dose or even low-dose estrogen therapy (55). The mysterious dual effects of estrogen on apoptosis may be due to altered global gene expression and to redirected intracellular signaling after long-term hormone deprivation (56, 57).

The rapid growth and division of tumor cells require sufficient nutrient supply from blood via angiogenesis. Angiogenesis involves several steps, including the degradation of existing vascular basement membrane, the proliferation and migration of endothelial cells into tubular structures in the tissue, and the formation of new matrix around neovessels. Estrogen induces blood vessel formation in the uterus during menstrual cycle. In breast cancer cells, E2 stimulates the secretion of interleukin 8 and vascular endothelial growth factor, both potent inducers of angiogenesis. In contrast, antiestrogen tamoxifen decreases tumor angiogenesis in ER-positive breast cancer (58–60).

Metastasis to distal organs is a major cause of death in cancer. In general, the E2-ERα pathway does not stimulate tumor metastasis. ER-positive breast cancers have less metastasis potential compared with more invasive ER-negative breast cancers. In addition, ER-positive breast cancer patients often have a better prognosis than do ER-negative breast cancer patients. Epithelial-mesenchymal transition is now considered the first step in metastasis. E2-ERα signaling upregulates epithelium-related transcription factors such as GATA3 and FOXA1 and promotes mammary epithelial differentiation along the luminal/epithelial lineage (61). Estrogen signaling inhibits de novo synthesis of RelB, a subunit of NF-κB that represses E-cadherin expression and promotes cell migration and invasion (62). Moreover, forced expression of ERα in ERα-negative breast cell lines induces the epithelial phenotype and reduces invasiveness, whereas knockdown of ERα in ERα-positive breast cell lines leads to the mesenchymal phenotype and increased invasiveness (63). Interestingly, the upregulation of several ER coregulators, such as AIB1 (amplified in breast cancer 1), SRC-1, and PELP1 (proline, glutamate, and leucine-rich protein 1), promotes breast cancer invasiveness and metastasis (64). For example, AIB1 is encoded by a well-known oncogene that is often overexpressed in breast cancer. In addition to stimulating cell proliferation, AIB1 stimulates tumor cell motility and invasion. The Drosophila AIB1 homolog
is crucial for ovarian border cell migration and invasion during oogenesis (65). Elevated AIB1 expression is significantly correlated with seminal vesicle invasion and lymph node metastasis of prostate cancer (66). AIB1 directly regulates the transcription of matrix metalloproteinase (MMP)-2 and MMP-13 through its coactivation of AP1 and PEA3/ETV4 (ETS translocation variant 4) (66). AIB1 can promote cancer cell motility while remodeling the local microenvironment to facilitate tumor cell invasion into the adjacent microenvironment (67). Instead of interacting directly with the estrogen-ER complex, AIB1 is thought to participate in other signaling transduction pathways or to act as a coregulator of other transcription factors in promoting tumor metastasis (64).

INTERFERING WITH ESTROGEN ACTION IN CANCER TREATMENT

Antiestrogen strategies remain the primary breast cancer treatment because at least 70% of breast cancers are classified as ER-positive breast cancers. As such, modulation of estrogen signaling has been a mainstay of breast cancer treatment for more than a century. Prior to the development of antiestrogen therapies, surgical oophorectomy was the chosen method of eliminating estrogen function in premenopausal breast cancer patients. Tamoxifen was introduced in the 1970s, followed by the rapid development of selective estrogen receptor modulators (SERMs) and AI. With the success of these antiestrogen drugs, the American Society of Clinical Oncology no longer recommends ovarian ablation in systematic breast cancer therapy (68).

SERMs are a group of synthetic chemical compounds that are structurally related to estrogens and that bind to and modulate ER function in different tissues (2). The FDA has approved three SERMs: raloxifene, toremifene, and tamoxifen (Figure 1a). Raloxifene, a second-generation SERM, is used mainly to treat or prevent osteoporosis. Toremifene has antiestrogenic activity in the mammary gland. It is presently used in postmenopausal women with metastatic breast cancer (69). In 2009, the FDA approved toremifene to reduce fractures in men who had prostate cancer and who were receiving androgen deprivation therapy.

Fulvestrant is representative of yet another type of ER modulator that promotes ER turnover in cells (70, 71). Fulvestrant has been approved as a third-line treatment for ER-positive metastatic breast cancer in postmenopausal women.

Tamoxifen was the first drug developed to target ER function and, due to its efficacy and low price, remains the preferred choice for treating hormone-sensitive breast cancers. Although tamoxifen was initially used only in the treatment of advanced breast cancer in postmenopausal women, it is now used as an adjuvant therapy to reduce the risk of breast cancer recurrence after surgery in both pre- and postmenopausal women, as well as in women at high risk for breast cancer. A large STAR (Study of Tamoxifen and Raloxifene) clinical study compared the efficacies of tamoxifen and raloxifene in reducing the incidence of breast cancer. Finished in 2006, STAR concluded that raloxifene is as effective as tamoxifen, although there were statistically insignificantly fewer uterine cancers and blood clots in women taking raloxifene (72). Tamoxifen greatly reduces the rate of breast cancer recurrence and has contributed significantly to the 25–30% decrease in breast cancer mortality in the past two decades (73).

Tamoxifen acts as an ER antagonist in breast cancer cells. Crystal structure studies indicate that the ER LBD interaction surfaces are composed of amino acid residues belonging to α-helices 3, 4, 5, and 12. When the ERα LBD is complexed with E2, α-helix 12 is positioned over the ligand-binding pocket and forms an interaction surface for the recruitment of coactivators. In contrast, when either the ERα LBD or the ERβ LBD is liganded with tamoxifen or raloxifene, α-helix 12 is displaced from its agonist position and occupies the hydrophobic groove formed by α-helices 3, 4, and 5, causing α-helix 12 to block the coactivator interaction surface (2, 74).
acting as an ER antagonist in the breast, tamoxifen displays partial estrogenic effects in other target tissues, such as the bone, uterus, and cardiovascular system. These partial estrogenic actions have beneficial effects on the bone and the cardiovascular system in postmenopausal women. However, tamoxifen has been associated with an increased incidence of endometrial cancer. Differential expression of ER coregulators may contribute to the tissue-specific response to tamoxifen (75). Comparison of gene expression profiles in cancerous versus normal endometrial epithelial cells treated with tamoxifen indicates that PAX2, one of the PAX family of transcription factors, is the key player in tamoxifen-stimulated endometrial cancer (76). Finally, because ERβ does not stimulate breast or uterine tissues in response to E2, selective ERβ agonists may be beneficial in treating breast cancer (16, 17).

Breast cancer tissues express aromatase and produce higher levels of estrogen than do noncancerous cells (7). Because aromatase catalyzes the final and rate-limiting step in estrogen biosynthesis, inhibitors of this enzyme are an effective targeted therapy for breast cancer. As discussed above, AIs are used mainly to treat breast cancer in postmenopausal women. AIs have also been combined with radiation therapy and/or chemotherapy to treat recurrent, metastatic, and high-risk endometrial cancer. There are two types of AIs. Type I inhibitors have a steroid-like structure similar to that of androstenedione, the substrate of aromatase, and the covalent binding between these inhibitors and aromatase irreversibly inhibits the enzyme. In the case of type II inhibitors (or nonsteroidal AIs), binding between the inhibitors and aromatase is noncovalent, and androgen can compete with these agents and relieve the inhibitory effect on aromatase function. Three AIs—anastrozole, letrozole, and exemestane—are FDA approved and used in clinical practice. Among these agents, exemestane is a type I inhibitor, and anastrozole and letrozole are type II inhibitors. AIs can be used after five years of tamoxifen treatment, as recommended by the National Comprehensive Cancer Network breast cancer guidelines. AIs are often effective in tamoxifen-resistant breast cancer (7) because AIs reduce estrogen biosynthesis, whereas tamoxifen antagonizes estrogen action within breast cancer cells.

**EPIGENETICS AND ENDOCRINE RESISTANCE IN CANCER**

Although antiestrogen drugs have proven to be the most successful targeted cancer therapy in modern medicine, drug resistance remains a major challenge in the treatment of breast cancer, even with positive ER expression. The National Cancer Institute estimates that more than 200,000 Americans are diagnosed with breast cancer every year. More than 65% of breast tumors express ERα, but fewer than half of those tumors respond to antiestrogen therapy (77). Moreover, a significant number of patients who initially respond to antiestrogen agents eventually become refractory and exhibit a more aggressive disease with a poorer prognosis. Numerous studies have tried to elucidate the underlying mechanisms of both intrinsic and acquired endocrine resistance, and various approaches have been proposed to enhance the antitumor efficacy of tamoxifen and AIs. Fighting endocrine resistance remains a significant challenge in the field of breast cancer research. Recently, several comprehensive reviews have focused on the possible underlying mechanisms of endocrine resistance in breast cancer (19, 28, 78). Most research in this area has focused on tamoxifen resistance, given its widespread use and the existence of tamoxifen-resistant cell lines and animal models for mechanistic studies.

In general, resistance to antiestrogen therapy can be caused by genetic or epigenetic deregulation of all components in the estrogen signaling pathway. The prodrug tamoxifen is first converted to its active metabolite, endoxifen, by the hepatic drug-metabolizing enzyme cytochrome P450 2D6 (CYP2D6). Polymorphisms in the CYP2D6 gene alter the efficiency of the tamoxifen-to-endoxifen conversion and can greatly affect the clinical response to tamoxifen. Although a strong
link between CYP2D6 polymorphisms and tamoxifen response is unestablished, genetic screening for CYP2D6 variants may eventually prove useful for guiding the selection and dose of tamoxifen (79).

Ligand binding to ER recruits a panel of coactivators or corepressors; variation in coregulator expression greatly affects the composition and output of the large ligand-ER complex. Consequently, abnormal expression of ER coregulators, such as AIB1 and SRC-1, or of components of other signaling pathways, such as ERBB2, can contribute to tamoxifen resistance (19, 80). Genetic mutation, gene amplification, gene fusion, and epigenetic deregulations can alter the expression of coregulators. For example, both AIB1 overexpression by gene amplification and certain AIB1 polymorphisms cause tamoxifen resistance in patients (67, 81). Overexpression of the specific splicing variant AIBΔ3 can also affect responsiveness to antiestrogen therapy because this variant appears to be more potent than wild-type AIB1 in provoking in vivo pathological changes in the mammary gland (67). Dysregulation of these ER targets is thought to oppose the antagonistic effects of tamoxifen and to contribute to drug resistance. For example, overexpression of cyclin D1 or underexpression of Bcl-2 is associated with many tamoxifen-resistant breast cancer patients (19). Large-scale genomic studies have identified gene signatures that predict therapeutic responses to endocrine therapies, albeit with inconsistent results (19). In addition, noncoding RNAs such as miR-451 may also play a role in tamoxifen resistance (82, 83). The studies suggest that targeting specific genes associated with resistance may increase the efficacy of tamoxifen, as shown for trastuzumab, an anti-ERBB2 drug used as an adjuvant therapy in ER-positive, ERBB2-positive breast cancer (19, 28).

Efforts to define epigenetic mechanisms underlying endocrine resistance have been made (80, 84). Tamoxifen resistance emerges two to three years after the initial drug administration. This time factor suggests that defects in epigenetic marks, rather than primary DNA mutations, likely play a role in the progression of drug resistance. Epigenetic events can impact chromatin by direct DNA methylation at cytosine on CpG sequences, PTMs of histones, and the presence of histone variants and nonhistone chromosomal proteins. All these epigenetic factors greatly influence local gene expression, and the reversibility of epigenetic modifications also makes them druggable. Environmental cues influence epigenetic regulation, and it is tempting to speculate that long-term treatment with tamoxifen or other antiestrogen agents may induce epigenetic changes in breast cancer and endometrial cancer cells. Epigenetic factors may also contribute to endocrine resistance in breast cancer, with loss of ER expression attributed to promoter hypermethylation of the ESR1 promoter (16). Treating ER-negative breast cancer cells with the DNA methyltransferase (DNMT) inhibitor AZA restores ER expression and sensitizes these cells to tamoxifen treatment (85). ESR1 is not the only gene that is subjected to abnormal methylation in tamoxifen-resistant breast cancer cells. Candidate gene and unbiased approaches using high-throughput DNA methylation profiling revealed different promoter methylation signatures predictive of tamoxifen responsiveness (85, 86). Unfortunately, other than PITX2 (paired-like homeodomain 2), a consensus of relevant markers affected by methylation has yet to emerge. In this respect, DNA methylation mapping by whole-genome bisulfite sequencing may be useful in identifying the relevant markers of endocrine resistance.

Although histone modifications also affect global or individual gene expression, less is known about how abnormal histone modifications contribute to endocrine resistance in breast cancer. Lysine acetylation of histone H3 and H4 often leads to an open chromatin structure and therefore correlates with transcriptional activation. Histone acetylation is catalyzed by HATs and is removed by histone deacetylases (HDACs). Mutations or abnormal expression of several HATs, such as CBP, p300, and Hbo1, or of HDACs, such as HDAC1, HDAC2, HDAC3, and HDAC6, are associated with breast cancer progression or prognosis (85, 87). Histone lysine methylation can cause either transcriptional activation or transcriptional repression, depending on the specific
residue and methylation state (mono-, di-, or trimethylation). The methyltransferase EZH2 catalyzes trimethylation of H3K27, and overexpression of EZH2 has a significant correlation with advanced stages and poor prognosis of many types of cancer, including that of the breast. Expression of other histone methyltransferases or demethylases is often altered in different stages of breast cancer (80, 87); such expression presumably affects global or local chromatin, alters gene expression, and ultimately leads to tumor progression or drug resistance.

Although the underlying mechanisms for deregulated histone modifications in endocrine resistance have yet to be defined, many laboratory and clinical studies have evaluated specific enzyme inhibitors in breast cancer treatment. Combining AZA and the HDAC inhibitor TSA can greatly restore ERα expression and sensitize ER-negative breast cancer cells to tamoxifen treatment (88). A recent study demonstrates that addition of the HDAC inhibitor vorinostat (VPA) to ER-positive breast cancer cells changes the normal response to tamoxifen from growth arrest to apoptotic cell death. Thus, HDAC inhibitors may increase the efficacy of tamoxifen treatment (77). In this regard, the FDA has approved VPA for the treatment of cutaneous T cell lymphoma, and at least 80 trials are under way to assess the clinical usefulness of other HDAC inhibitors in hematological and solid malignancies.

**FUTURE PERSPECTIVES**

Theoretically, any cell that expresses ER could respond to estrogen and other ER modulators. Sustained estrogen exposure or deregulated estrogen signaling is associated with many types of cancer, including those of the breast, endometrium, ovary, prostate, lung, and colon. Although tamoxifen and AIs have been used mainly to treat breast cancer and certain types of endometrial cancer, we do not yet know whether these antiestrogen therapies could be used to treat other cancers. A recent report indicates that E2 stimulates ER-positive lung adenocarcinoma cell growth but inhibits ER-negative lung adenocarcinoma cell growth (89). Whether interfering with estrogen signaling benefits ER-positive lung cancer patients remains to be determined. Developing new ERα-specific SERMs is still one of the major strategies for treating tamoxifen-resistant breast cancer and other estrogen-related diseases. Increasing evidence indicates that changing the ratio of ERα/ERβ expression may play a pivotal role in tumor development and progression. Given that ERβ acts as a tumor suppressor to inhibit cell proliferation, cell growth, and angiogenesis, an ERβ agonist could benefit ERβ-positive cancer patients. Future efforts to target ERβ offer an alternative approach to finding new ERα-specific SERMs. Finally, identifying global gene signatures that predict the antiestrogen responsiveness of specific tissues could better guide clinical applications of single and combined drug therapies in the fight against estrogen-responsive cancers.

**SUMMARY POINTS**

1. Estrogen is a well-established risk factor for many types of cancer, particularly breast and endometrial cancer.

2. The primary mediators of estrogen action are estrogen receptor (ER)α and ERβ, which function as ligand-induced transcription factors to regulate the expression of diverse genes.

3. The oncogenic effects of estrogen are due mainly to ERα-mediated transcriptional activation of genes that promote cell proliferation or alleviate apoptosis.
4. Selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) are the two main antiestrogen therapies.


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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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