Review on the Role of Estrogen Receptors in Breast Cancer

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Summary
Estrogens play a significant role in regulating the growth and differentiation of normal, premalignant and malignant cell types, especially breast epithelial cells, through interaction with two nuclear estrogen receptors (ER alpha and ER beta). Estrogen receptor (ER) plays a central role in mediating the effects of endogenous hormones and endocrine therapy has been an integral treatment for patients with hormone-dependent breast cancers. ER is targeted either directly by selective estrogen receptor modulators and pure antagonists or indirectly by aromatase inhibitors that block estrogen production. A significant number of ER-positive patients, however, fail to respond to therapy or develop resistance over time. ER also serves as a prognostic marker for responsiveness to endocrine therapy. In this review, we present a brief overview of the role of ERs in breast carcinogenesis, including cancer progression to metastasis and of their clinical importance in the prevention, treatment and prognosis of the disease.

Introduction
Breast cancer is one of the most common and most frequently diagnosed cancers in women both in the developed and less developed world. It is also a leading cause of cancer death in women. According to the American Cancer Society about 1.3 million women will be diagnosed with breast cancer annually worldwide and about 465,000 will die from the disease. In the United States, 232,340 new cases of invasive breast cancer were diagnosed in women in 2013 with 39,620 breast cancer deaths[3]. In the developing world, although definitive data is not available, it is widely accepted that the incidence of breast cancer is increasing due to increase life expectancy, increase urbanization and adoption of western lifestyles. In 2008, the number of new cases of breast cancer in women from Africa was estimated to be 92,600 cases with alarming 54% (50,000) deaths from breast cancer[7]. In Ethiopia, breast cancer is the second most common cancer among women and it is estimated that around 10,000 Ethiopian women have breast cancer with thousands of more cases unreported[12].

Breast cancer is no longer seen as a single disease but rather a multifaceted disease comprised of distinct biological subtypes with different prognostic and therapeutic implications. One major way of defining your type of breast cancer is whether or not it is Estrogen receptor (ER)-positive. About 75% of all breast cancers are ER+ meaning they grow in response to the hormone estrogen. Such breast cancers are designated hormone-receptor positive and are likely to respond to endocrine therapies.

Estrogen effects are exerted through two types of specific receptor: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ)[8]. These nuclear receptors are ligand-dependent transcription factors that mediate the biological effects of estrogens and anti-estrogens. Estrogen, 17β-estradiol (E2), plays a prominent role in mediating the maturation, proliferation, differentiation, apoptosis, inflammation, metabolism, homeostasis, and brain function and influences the growth and development of breast cancer. Estrogen receptors act mainly by regulating the expression of target genes whose promoters contain specific sequences called estrogen-responsive element (ERE). After ERE-binding of ligand-bound ER dimers, modulation of transcription occurs via interaction with co-activators or co-repressors. These complexes play an important role in the recruitment of transcriptional machinery, the modulation of chromatin structure, and in the regulation of ER target-gene expression[16].

Estrogen receptors have evolved to be the most effective target for breast cancer therapy since endogenous estrogens are thought to play a major role in breast cancer development. Interactions between estradiol (E2) and the ER can be blocked using a variety of agents. Selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene are competitive inhibitors of E2 at the estrogen receptor and display agonist or antagonist behavior depending on the tissue. While ER can be removed and degraded by pure antiestrogens such as fulvestrant. In addition, estrogen production can be significantly blocked by inhibiting the conversion of steroidal precursors to estrogen using aromatase inhibitors such as anastrozole and letrozole[11].
The value of ERα status as an independent prognostic variable has been previously reported and overexpression of ERα is a well-established prognostic factor in breast cancer patients. Generally, breast tumors that express the ERα-positive behavior in a fundamentally different fashion than ER-negative (ER-) tumors with regard to their response to hormonal therapies, given that outcomes are often favorable in ER-positive breast tumors treated by endocrine therapy alone[2]. Moreover, more than 90% of lobular breast carcinomas are ER-positive, while medullary and inflammatory carcinomas are predominantly ER-negative and are often associated with aggressive disease. This review briefly summarizes the role ER plays in breast tumor growth and invasion and its potential value in cancer therapy and prognosis.

Estrogen receptors (ERs) in normal mammary gland physiology and in malignant mammary tissues

Estrogen receptors are essential for mammary gland morphogenesis and physiological events such as puberty and pregnancy. Analysis of estrogen receptor proteins in rat mammary gland have shown that both ERs are expressed, and the results from ER-α-KO and ER-β-KO mice reveal that ER-α is necessary for mammary gland development. It has been suggested that ER-β co-expression with ER-α represses ER-α function and may contribute to the insensitivity of the mammary gland to estrogens during lactation [23]. Both receptors are expressed next to one another in many tissues, including the central nervous system, the cardiovascular system, the urogenital tract, the breast, and the bone. In the uterus and mammary gland, ER-α is an important estrogen receptor and much more frequently expressed than ER-β. In addition, ER-α is also present in liver, while in the gastro-intestinal tract it is only ER-β [8].

The expression of ER-α and ER-β were studied by immune-histochemistry in normal and premalignant tissues. The percentage of ER-α-positive cells is generally low (10–20%) in normal resting mammary glands and increases in proliferative benign disease, particularly when associated with atypia and in low-grade ductal carcinoma in situ (DCIS). This has suggested that an elevated receptivity to estrogens in these tissues is involved in their higher risk of tumorigenesis. In contrast to ER-α, the ER-β level decreased from proliferative ductal hyperplasia to DCIS, whereas in high-grade DCIS, both ER levels were low or absent. The analysis of knockout mice has provided a framework in which to study the potential functions of ER alpha and ER beta in human target tissues. Phenotypes of Alpha estrogen receptor knock out (ER-α KO) mice have pointed toward the importance of ER alpha in the uterus and mammary gland of females. In addition, Beta estrogen receptor knock out (ER-β KO) mice have suggested an important function for ER beta in the ovary in females and in the prostate gland in males. These laboratory studies in mice naturally advance the study of the complex role of the individual ERs in human cancer [19].

Molecular mechanism of ER signaling pathways

Emerging evidence suggests that there are several distinct pathways by which estrogens and ERs may regulate biological processes. The classical (direct) pathway includes ligand activation and a direct DNA binding to estrogen response elements (ERE) before modulation of gene regulation. The tethered pathway includes protein-protein interaction with other transcription factors after ligand activation, and thereby gene regulation is affected by indirect DNA binding. A third mechanism, also called non-genomic with rapid effects, is not as well understood as the genomic mechanism but has been observed in many tissues. The ligand activates a receptor, possibly associated with the membrane; either it is a classical ER, an ER isoform (or a distinct receptor or, alternatively, a signal activates a classical ER located in the cytoplasm. After this rather unclear event, signaling cascades are initiated via second messengers (SM) that affect ion channels or increase nitric oxide levels in the cytoplasm, and this ultimately leads to a rapid physiological response without involving gene regulation. The ligand-independent pathway includes activation through other signaling pathways, like growth factor signaling. In this case, activated kinases phosphorylate ERs and thereby activate them to dimerize, bind DNA, and regulate gene[17].

ER alpha and breast cancer progression

ER alpha was found to regulate a smaller set of genes that overlapped with ER alpha despite regulating many more genes not involved in estrogen signaling. Analysis of the microarray data from ER-regulated and estrogen related receptor (ERR)-regulated genes in MCF-7 cells by gene ontology (GO) showed that the majority of genes regulated by ER alpha are involved in energy metabolism, oxidative stress and detoxification as expected. Interestingly, ER alpha also induces vascular endothelial growth factor (VEGF), a highly angiogenic factor. Further studies demonstrated that ER alpha-dependent activation of VEGF mRNA expression occurs in several different breast cancer cell lines suggesting that ER alpha promotes tumor cell growth by stimulating VEGF expression. ER alpha functions as a key modulator of intratumoral estrogen production in human breast carcinoma by stimulating the expression of the androgen-estrogen key converting enzyme, aromatase via tumor specific promoter usage[27]. These studies provide a basis for highly expressed ER alpha to be considered an overall negative phenotype of breast cancers.
ER beta and breast cancer progression

The second receptor, ER beta, has likewise been detected in human breast cancers but its role in breast cancer growth and development has not been delineated. ER-beta may contribute to hormonal sensitivity and resistance. However, several studies indicated that the ER beta RNA level was decreased in invasive breast cancer tissues compared with the adjacent normal mammary gland. The mechanism and role of the decrease in ER beta in carcinogenesis are unknown[18]. There is no clear evidence that ER beta expression is linked to clinical parameters in breast cancer. This may be due to difficulties in accurately quantifying ER beta protein levels using existing reagents and techniques[9]. While estrogen treatment of ER alpha-positive breast cancer cells stimulates proliferation, exogenously introduced ER beta in some studies suppresses ER alpha-induced proliferation and transcriptional activity while also inducing independent transcriptional and functional changes. Related to these anti-proliferative effects, it has also been reported that ERβ-positive tumors may respond more favourably to tamoxifen, and ER-β agonist treatment of ERα-positive breast cancer cell lines appear to enhance their sensitivity to tamoxifen. Re-introduction of ER β in more invasive ER α-negative breast cancers can, however, increase cell proliferation. The body of data correlating ER β to both anti-proliferative and proliferative parameters suggests a bifurcated role for ER β breast cancer biology, but the exact function of ERβ in tumourigenesis and disease progression remains to be determined[13].

ERs as prognostic markers of primary breast cancers

The use of biomarkers ensures breast cancer patients receive optimal treatment. Established biomarkers such as ER have been playing significant roles in the selection and management of patients for endocrine therapy. The prognostic value of ER-alpha and ER-Beta are discussed below.

ER alpha expression as a prognostic marker in breast cancer

Over expression of ERα is a well-established prognostic factor in breast cancer patients. Generally, ERα-positive breast cancers are associated with slow tumor growth, lower histology grade, DNA diploidy, and thus a better overall prognosis [1]. As a result, ER alpha has been utilized to predict response to hormonal therapy, both in the adjuvant setting and for advanced breast cancer. Tumors that express ER alpha have the greatest benefit from hormonal therapy. However, ER alpha re-expression in an ER alpha -negative cancer cell is not sufficient to restore the ER alpha-positive phenotype, particularly in terms of therapy response and the pattern of gene expression. In other studies conducted on ERs by measuring mRNA levels in primary breast tumors versus normal mammary gland epithelial cells from breast reduction surgery, it has been revealed that ER alpha is highly expressed in a subset of tumors with elevated levels of ErbB2, an indicator of aggressive tumor behavior and nonfunctional ER alpha[1]. Additional studies using an immune-histochemistry approach combined with reverse transcription PCR supported findings that ER alpha expression in breast carcinoma could be associated with an increased risk of recurrence and an adverse clinical outcome[28].

ER beta expression as a prognostic marker in breast cancer

Since the majority of ER present in breast tumors is ER alpha, the biological relevance of ER beta as a prognostic marker of breast cancer remains uncertain. However, the prognostic value of ER-β in breast cancer has been evaluated in some previous studies, and the majority has provided evidence of ER-β being a beneficial factor. One study indicated that ER beta is a good prognostic indicator for breast cancer. Expression of ER beta was associated with better survival in patients receiving adjuvant tamoxifen[15]. Another study showed that ER beta is associated with negative axillary node status and low grade tumors[10]. In addition, ER beta cases had a better disease free survival rate and levels of ER beta were decreased in proliferative pre invasive tumors[22]. These studies suggest a protective role for ER beta in breast cancer. In contrast, evidence also suggests that ER beta is a poor prognostic indicator. Tumors that expressed both ER alpha and ER beta were node positive and of a higher grade. ER beta mRNA levels were also elevated in tumors that displayed tamoxifen resistance[26]. Overall, the majority of studies suggest that the presence of ER beta is a good prognostic marker for breast cancer.

Targeting estrogen for breast cancer therapy and prevention

The development of therapeutics for ER-expressing breast cancers has been one of the great clinical advances of the past 50 years and has served as a paradigm for the development of targeted therapies in oncology. Presently, anti-estrogen therapies are a mainstay of treatment of ER-positive breast cancers. The ER is an important target to develop drugs for the treatment and prevention of breast cancer. As discussed below, this can be achieved by blocking the production of estrogen, inhibiting the conversion of steroidal precursors to estrogen using aromatase inhibitors, by using SERMs such as tamoxifen and raloxifene as competitive inhibitors of estrogen action and/or by the removal and degradation of the ER by pure anti-estrogens such as fulvestrant[19].

Tamoxifen and other adjuvant therapies of breast cancer

Selective estrogen receptor modulators (or SERMs) bind ERs but have a mixed agonist/antagonist profile. Tamoxifen and raloxifene are well-known first and second generations of SERMs, respectively. Current
treatment strategies have shown that, 5 years of adjuvant tamoxifen treatment is beneficial in pre- and postmenopausal women with ER-positive tumors. In addition, tamoxifen can be used for the prevention of breast cancer. The activity of tamoxifen is dependent on circulating levels of E2, which are high in premenopausal women and low in postmenopausal women. Tamoxifen is an anti-estrogen in the breast, and decreases low density lipoprotein cholesterol levels in postmenopausal women. Tamoxifen treatment decreases bone density in premenopausal women but increases bone density in postmenopausal women[20]. In premenopausal women, alternatives to tamoxifen include ovarian suppression with luteinizing hormone releasing hormone agonists, ovarian ablation and progestins. A recent study involving patients with metastatic disease indicated a higher survival with the combination of tamoxifen and ovarian suppression versus tamoxifen alone[20]. In addition, Wu L et al showed that arzoxifene and 4-hydroxytamoxifen (4OHT) can inhibit specifically the repopulation of ER+ MCF-7 breast cancer cells during courses of weekly treatment with methotrexate. Most recently, they further confirmed that combined treatment with arzoxifene given between cycles of paclitaxel can inhibit repopulation of MCF-7 breast cancer xenografts[29]. They proposed that scheduling of short-acting anti-estrogenic agents between courses of adjuvant chemotherapy for human breast cancer has potential to improve the outcome of treatment. Additionally, the increased etoposide cytotoxicity by tamoxifen as compared to cells treated with either drug alone was observed in breast tumor HTB-14 cells expressing ER, which was accompanied with enhanced inhibition of protein kinase C and insulin-like growth factor II[21]. The pure anti-estrogens, also called selective estrogen receptor downregulators like fulvestrant. Unlike tamoxifen, fulvestrant is a pure antagonist of estrogen-regulated gene expression that could down-regulate ER expression without any concomitant rise in other growth signal pathways. Clinical studies have demonstrated that this agent is active in second-line therapy after tamoxifen failure but as yet it has been shown no efficacy and limited safety in premenopausal women.

Aromatase inhibitors

One strategy to inhibit the activation of estrogen/ER pathway is to block the conversion of estrogen precursors into estrogen by aromatase inhibitors (AIs)[25]. Currently, third generation aromatase inhibitors, such as the non-steroidal agents anastrozole, letrozole and the steroidal agent exemestane, have been introduced into the market as endocrine therapy in postmenopausal patients, either alone or as part of multiple hormonal therapies[6]. These very potent inhibitors of aromatase decrease estrogen levels below the level of detection of most clinical assays. In the first line setting, anastrozole and letrozole, are now considered more effective than tamoxifen as first-line therapy for metastatic breast cancer in postmenopausal women, regardless of whether the patients have received tamoxifen as adjuvant therapy. In addition to the above AIs, cyclooxygenase (COX) inhibitors also decrease aromatase mRNA expression and enzymatic activity[4]. However, the use of aromatase inhibitors is at the expense of accelerated bone loss, and strategies to minimize this side effect are under investigation[24]. A recent study by Chen D et al showed that the combination of paclitaxel with exemestane produced additive antitumor effect in cultured human breast cancer cell lines. Interestingly, this additive effect was independent of ER alpha expression, but dependent on the presence of androstenedione. The effects of AIs on sensitivity of ER- positive breast tumors to chemotherapy remains unclear and need to be further investigated.

Tamoxifen resistance and Second therapy for anti-estrogen-resistant tumors

A significant proportion of ER-positive tumors are resistant to tamoxifen therapy either at the first treatment or after an initial positive response. Tamoxifen resistance could also be explained by ER mutations, co-repressor expression and recruitment, or interactions with other signaling pathways. Additionally, a number of nonspecific mechanisms may contribute to the tamoxifen resistant phenotype. The mechanisms may include events that limit the intracellular availability of tamoxifen, such as binding to other proteins, partitioning into lipophilic membrane domains, altered transport into or out of the cell, the development of oxidative stress or the conversion of tamoxifen to other metabolites. Other mechanisms include over expression of growth factors, increased angiogenesis or heterogeneity in the tumor cell population[14]. The cross-talk of estrogen receptors with growth factor signalling pathway is well demonstrated and appears implicated in breast cancer progression and tamoxifen resistance. Over expression of growth factor receptor causes resistance to tamoxifen through protein kinase activation. Moreover, preclinical studies indicated that inhibitors of growth factor tyrosine kinase have the potential to delay or even reverse tamoxifen resistance. Overall, there are numerous potential mechanisms that may contribute singly or in combination to the development of drug resistance. Despite the possibility of drug resistance, there are potential treatments after the development of tamoxifen resistance. These include the use of aromatase inhibitors to block the production of estrogen as well as pure antiestrogens to degrade the ER or to prevent the growth of tamoxifen-resistant tumors. Fulvestrant was the first pure antagonist tested in tamoxifen-resistant breast carcinoma[14].

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Conclusion

The estrogen receptor (ER) plays a central role in the hormone action. Experimental and clinical data have demonstrated the importance of ER in the development and progression of breast cancer and this has led to its becoming a major target for breast cancer treatment. The efficacy of anti-estrogen treatment to inhibit the growth of ER-positive breast cancer cells has been extensively documented. Current successes in the treatment of hormone-dependent breast cancers still leave room for significant improvements in the specificity and efficacy of current endocrine therapeutic approaches and in overcoming resistant tumors. Accumulating insights regarding estrogen signaling and mechanisms of action of ligands and ER provide opportunities for the development of novel markers, targets, and therapeutic strategies. It should also be noted that there is an indirect hormone therapy using aromatase inhibitors. Aromatase inhibitors such as formestane, anastrozole or letrozole inhibit the activity of the aromatase enzyme to block the synthesis of estrogens. This method becomes more and more important in adjuvant hormone therapy for treating breast cancer. The identification of the factors that inhibit the invasiveness of ER alpha-positive cells would be a useful step in the development of new therapeutic targets to cure the most aggressive ER alpha-negative tumors.

References