

Anti-hormones: Mechanism and Use in Treatment of Breast Cancer

SUMI DINDA

LEARNING OBJECTIVES:

1. Define the roles of estrogen and progesterone in breast and uterine cells.
2. Define hormone-dependent and independent tumors.
3. Describe the classification and main functions of anti-hormone therapy.
4. Explain the mechanism of aromatase inhibitors, ERDs and SERMs.
5. Explain the differences between the mechanisms of tamoxifen and raloxifene.

ABSTRACT: Breast cancer is the second-leading cause of cancer-related death in women. Recently, new drugs are being developed based on the molecular mechanisms of receptors, tumor suppressor genes, monoclonal antibodies, tumor markers and anti-hormone therapy. Anti-hormone therapy is used in the treatment of hormone-dependent breast tumors. Among the anti-hormone therapies, a substantial amount of research has been focused on the development of the ideal selective estrogen receptor modulator to treat metastatic breast tumors and to prevent breast cancer in high risk women.

INDEX TERMS: Cancer stem cells, leukemia stem cells, cancer therapeutics, stem cell signaling pathways

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INTRODUCTION

Breast cancer is the most commonly diagnosed malignant disease among women and, after lung cancer,

the second-leading cancer-causing death in North America. The survival rates of breast cancer have steadily increased with earlier detection, surgery, chemotherapy, radiation therapy and anti-hormone therapy. The advancement of molecular targeted drugs, along with anti-hormone therapy, has provided clinicians more ammunition to fight breast tumors in advanced stages.¹ This article focuses on the molecular mechanism of different anti-hormone therapies on breast cancer in post-menopausal women. Estrogen and progesterone are major sex hormones known to play an important role in the normal growth and differentiation of breast and uterine cells. These hormones bind to their respective nuclear receptors and cause the transcription of different genes that carry the signal for these hormones. Estrogen is a proliferative hormone and progesterone is a differentiating hormone in the uterus, while estrogen and progesterone both have proliferative effects on breast epithelium cells. Estrogen is also known to induce progesterone receptors in different tissues through different receptor-mediated gene action.

Hormone therapy medicines are whole-body (systemic) treatments for hormone-receptor-positive breast cancers. HER2 is a protein that controls cell growth; breast cancer cells express high levels of HER2. If a cancer has receptors for either estrogen or progesterone and is HER2 negative, it is considered hormone-receptor-positive or a hormone dependent tumor. In contrast, a cancer that has no receptors for estrogen and progesterone but is HER2 positive is known as a hormone-independent tumor. Studies show that about 80% of breast cancers are estrogen-receptor positive; 65% of estrogen-receptor-positive breast cancers are also progesterone-receptor-positive.² Anti-hormone therapies target the action of estrogen and may indirectly affect progesterone actions. Anti-hormone therapy medicines are used to lower the recurrence risk of early-stage hormone-receptor-positive breast cancer, lower the risk of hormone positive breast cancer in women who are at higher risk of carrying a mutant form of tumor

suppressor genes BRCA1, 2 & 3 but have not shown any occurrence of breast cancer, and shrink or slow the growth of advanced-stage or metastatic hormone-receptor-positive breast cancers.

Anti-estrogen hormone therapies include aromatase inhibitors, *estrogen receptor down-regulators* (ERDs) and *selective estrogen receptor modulator* (SERMs). These drugs either affect estrogen production or block estrogen action at the receptor level. In contrast, anti-progesterone therapy, including the anti-progesterone drug RU 486, is not cleared by the Food and Drug Administration (FDA) for the treatment of breast cancer in the USA.

Aromatase inhibitors stop the production of estrogen in post-menopausal women because they inhibit ovary production of estrogen. Aromatase inhibitors work by blocking the enzyme aromatase which turns the hormone androgen into small amounts of estrogen in the body.³ This means that less estrogen is available to stimulate the growth of hormone-receptor-positive breast cancer cells. Common aromatase inhibitor drugs include Arimidex, Aromasin and Femara, which can be taken orally.

Aromasin and Femara work through estrogen receptor mediated pathways. Nearly all of the effects of estrogens are mediated through their binding to nuclear proteins called *estrogen receptors* (ERs), transcription factors that regulate expression of estrogen-responsive genes.² Hormone binding activates the dimerization (conformational changes) of the ERs, which interact with the *hormone response element* on the DNA to activate or repress transcription (Figure 1).

There are two isoforms of ERs in the super family of nuclear steroid receptors. The classic ER which is now known as ER α contains 595 amino acids with a central *DNA binding domain* (DBD) along with a carboxyl-terminal *hormone binding domain* (HBD). The steroid receptors share common structural and functional characteristics at functional domains designated from A-F. The presence of heat shock proteins stabilizes the receptor configurations and the activation of the receptor is induced by phosphorylation via different protein kinases (Figure 2). Recently, ER β has been identified and is shorter than ER α by 65 amino acids (ER β has 530 amino acids). The DBD has the highest homology (95%) between ER α and ER β . Both ER α

and ER β are present in most tissues in the female body and the amount of ER α increases both in breast and uterine cancer whereas the ER β levels decrease.⁴ There are some differences in the A/B, D, and E/F domains and ER β lacks a large portion of carboxyl terminal F domain. The ER mediated gene transcription is initiated via two transactivation domains: a) AF-1 located in the amino terminal of A/B region and b) AF-2 located in the carboxyl terminal of E region (Figure 2).^{2,5} The AF-1 domain is hormone-independent whereas AF-2 domain is hormone-dependent. For maximal ER transcriptional activity both AF-1 and AF-2 are needed.

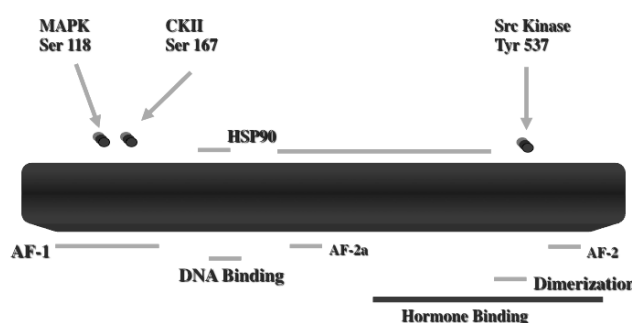


Figure 1. Structure of Estrogen Receptor (ER α).

ERDs are pure ER antagonists that bind the ER in competition with estrogen and down-regulate ER in human breast cancer cells. ICI182, 780 (Falodex) is a pure ER antagonist. This drug is cleared by the FDA to treat metastatic hormone-positive breast cancers in menopausal women and breast cancer that is resistant to other anti-estrogen treatments. It is given as an intramuscular injection. ICI182, 780 blocks ER transactivation from AF-1 and AF-2 domains. This drug may also impair ER dimerization and has been shown to induce ER degradation, decreasing the cellular ER concentration. Since ER is the major target for all anti-estrogens, reducing the cellular ER levels by ICI offers more effective treatment. In *in vitro* models, ICI has shown to be effective in blocking breast cancer cell proliferation. Our laboratory has shown that ICI182, 780 significantly inhibits hormone-dependent T47D and MCF-7 breast cancer cells in comparison to the same cells treated with estrogen. This suggests that ICI182, 780 induced apoptosis in these cell lines. Also, ICI182, 780 does not cross the blood-brain barrier and therefore reduces the risk of hot flashes, which is a common side effect of SERMs.⁶ This drug also possesses superior anti-tumor activity, which has been

demonstrated in a mouse model for normal breast tumors and tamoxifen-resistant breast tumors.

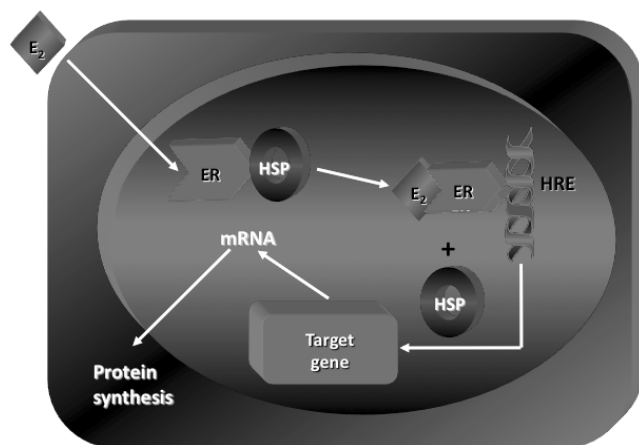


Figure 2. Hypothetical Model: Mechanism of Estrogen Action

SERMs block the effect of estrogen in one tissue and act as an anti-estrogen in other tissues. SERMs are divided into three major categories: triphenylethylene derivatives like tamoxifen, other non-steroidal compounds, and steroidal compounds that have more complete anti-estrogenic activity.² These oral medications are cleared by the FDA to treat breast cancer in pre- and post-menopausal women.

Tamoxifen, also known as Nolvadex, is approved for treating breast cancer in pre- and post-menopausal women. It was approved by the FDA many years ago to treat advanced stages of breast cancer. Tamoxifen blocks the transactivation of AF-2 in ER α that causes the antagonistic activity in breast tissue but activates the AF-1 domain of ER β in bones,^{7,8} the cardiovascular system, the vagina and the central nervous system with partial agonistic activity and predominantly estrogenic activity in the uterus, and liver.

Tamoxifen, like other SERMs, has also been shown to be cytostatic, blocking the cell proliferation at the G1 phase and may induce apoptosis in certain breast cancer cell lines.^{2,9,10} Due to the partial antagonistic and agonistic activity of tamoxifen in the CNS and vagina, patients on tamoxifen therapy often report troublesome hot flashes or vaginal dryness. The estrogenic activity of tamoxifen in the endometrium is linked to endometrial hyperplasia, low-grade endometrial tumors and thromboembolic phenomena. Despite all of the possible side effects, tamoxifen is one of the FDA-cleared drugs

used for breast cancer reduction in USA.¹¹

Toremifene, also known as Fareston, differs chemically from tamoxifen by one chlorine atom but acts similarly to tamoxifen. It is also cleared by the FDA to treat metastatic breast cancer in menopausal women. It has the same estrogenic effects as tamoxifen in the uterus.¹² Recently, toremifene has been associated with QT prolongation, which is an irregular heart rhythm that can lead to fainting, loss of consciousness, seizures, or sudden death.¹³

Raloxifene, also known as Evista, was originally developed to prevent osteoporosis in post-menopausal women and is now cleared by the FDA to treat and reduce the risk of hormone-receptor-positive breast cancer in post-menopausal women who are at higher risk for breast cancer due to the presence of mutated *BRCA1* and *BRCA2* genes. Raloxifene is also used to reduce the risk of breast cancer in post-menopausal women being treated for osteoporosis.

Raloxifene belongs to the benzothiophene class of SERMs; its structural differences from the triphenylethylene tamoxifen led to a slightly different tissue-specific estrogen agonist/antagonist profile.⁴ It has shown greater estrogen agonist activity in bone but has reduced estrogen agonist activity in the uterus due to the presence of Raloxifene response element in certain genes.¹⁴ In breast tissue, Raloxifene has pure ER antagonistic activity.

Recently, the National Cancer Institute-sponsored *Study of Tamoxifen and Raloxifene* (STAR) showed that raloxifene is as effective as tamoxifen in reducing breast cancer by approximately 50%, reducing the risk of uterine cancer by 36%, and reducing thrombosis by 29% compared with tamoxifen.¹⁵ The *raloxifene Use for the Heart* (RUTH) trial reported daily use of raloxifene resulted in side effects in the cardiovascular system of postmenopausal women.¹⁶ Some studies have reported side effects similar to those of tamoxifen such as hot flashes and an increased clotting risk resulting in stroke. A larger clinical study needs to be conducted with raloxifene in pre- and post-menopausal women to better understand its benefits and risks.

Our laboratory has been investigating the molecular mechanism of raloxifene analogue LY117018 on tumor suppressor proteins p53 and pRb in T47D breast cancer

cells. The product of p53 is a nuclear phosphoprotein which is essential to normal regulation of cell growth. Most cancer cells exhibit some mutated forms of p53. Abnormalities in p53 gene loci may play a significant role in breast cancer etiology.¹⁷ Inactivation of tumor suppressor gene retinoblastoma (*pRb*) has been studied in different cancers. The degree of *pRb* hyper-phosphorylation appears to be proportional to the loss of tumor suppressor characteristics.

The molecular mechanism is simplified and explained by a hypothetical model in Figure 3. Estrogen binds to ER α and activates the cyclin-dependent kinase cascade, which leads to the phosphorylation of *pRb*. Actions of p53 are known to be mediated by the product of a gene called p21, which is an inhibitor of cyclin-dependent kinases. This apparently prevents phosphorylation of retinoblastoma. The hyper-phosphorylated form of retinoblastoma is therefore responsible for the proliferative effects. When cells contain mutant p53, the expression of p21 is blocked. Therefore, estrogen's influence on *pRb* leads to its phosphorylation which is associated with cell proliferation. The binding of raloxifene to ER blocks the estrogen-induced proliferative effects of breast cancer cells either through the activation of wild-type p53 or through inhibiting the hyper-phosphorylated form of *pRb*. Our results demonstrate that raloxifene is as effective as ICI and more effective than tamoxifen in our system.¹⁸

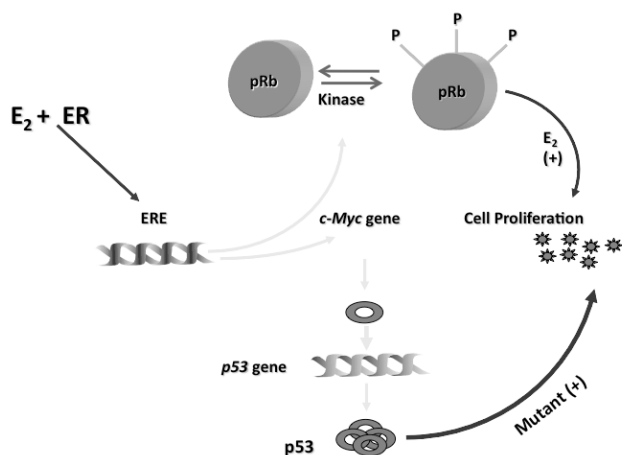


Figure 3. A hypothetical model for explaining the relationship between E2, LY 117018, p53 and pRb.

In conclusion, SERM analogs have significant clinical potential in the treatment of breast cancer as we expand our understanding of these compounds' molecular

mechanisms. Developing SERMs with fewer side effects than those currently in use is the ideal for the prevention of breast cancer in pre- and post-menopausal women. The optimal approach for future research includes delineation of the molecular mechanisms that are involved in ER signaling with tumor suppressor gene activation.


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