Estrogens are cyclopentafenanthene compounds. Estrogens are synthesized from cholesterol, like sex and adrenal hormones. Although most common estrogen found in humans is 17β-estradiol, estrone and estrol are synthesized at low levels in humans. Estradiol is involved in the development process of female sex organs, secondary sex characteristics, normal breast tissue and reproduction and regulation of menstrual cycle with interacting with other hormones. Estradiol has been shown to have a wide role in human physiology as well as in the pathogenesis of many diseases. As examples of these aforementioned pathological conditions; various types of cancer, osteoporosis, neurodegenerative and cardiovascular diseases, insulin resistance, Systemic Lupus Erythematosus (SLE), endometriosis and obesity can be mentioned. As a result of the conducted analyzes, it has been found that reproductive risk factors in breast cancer are mediated by hormones and estrogen plays the largest role in this process.

Estrogen Receptors (ERs)

Estradiol is passively diffuse to cytoplasmic and nuclear membranes due to their steroid structures and can interact with their own nuclear receptors. Estradiol is among the nuclear receptor family. Estradiol estrogen perform their most important physiological functions through two nuclear steroid receptors; ERα (NR3A1) and ERβ (NR3A2). ERα and ERβ act as a transcription factor and interact with other transcription factors and growth factor dependent kinases. Estradiol binding at both receptors initiates gene transcription via estrogen response elements (ERE's) in the target tissue and both receptors associated with different functions according to their distribution in different tissues. These two proteins are transcribed and translated from two separate genes located on different chromosomes and have tissue-specific expression profiles. If we look at overlapping expression distributions of these proteins; ER beta is
located predominantly at prostate, breast, bone, especially in the granulosa cells of the ovary, lung, central and peripheral nervous system whereas ER alpha is predominantly located in the pituitary gland, ovary strata, intestinal cells, uterus, kidney, adrenal, and mammary glands.\(^6\)

It has been revealed that ER alpha and ER beta have different biological activities from each other. Between these two ER isoforms, it has been determined that ER alpha has a stronger transcriptional activator function. It has been found that ER alpha exhibits this property at physiological concentrations of estradiol. Besides this, it has been reported that ER beta co-expression with ER alpha can suppress both the productivity and strength of hormone-stimulated response. Therefore, the interaction levels of these two isoforms with each other are important for determining the cellular estrogen sensitivity.\(^7\)

In 80% of breast carcinomas, ER alpha is expressed in high rates. It has been reported that the distribution of ER beta in breast cancer is exclusively nuclear and ER beta can be expressed many cell types (stromal, fibroblast, endothelial, etc.).\(^8\) It was also noted that the expression increased with tumor aggregation. Although ER beta expression in the breast was identified; ER beta function, ER beta’s clinical significance in carcinogenesis and value of ER beta in pathologic diagnosis of breast cancer are still not fully understood.\(^9\)

**Domain Structure of ER**

In Figure 1, the general structure of ER alpha and beta receptors is schematized. Domain structures in both receptors are shown individually. ER alpha/beta proteins contain common regions. A/B, C, D, E and F are among these common regions.\(^5, 6, 9\) Although the formations of these regions are independent of each other, it is emphasized that they are functional units that are in constant interaction with each other. When domain organization of these two receptors are examined, it has been reported that there is a high similarity in amino acid levels and also there are high rates of homology between specific domains of these two receptors.\(^6, 9\) The variable N-terminal region containing A/B domain is the part of the receptor which is responsible for the transcriptional activity of ER independent of a ligand and is responsible for activation of activation function-1 (AF-1). The highly conserved C domain structure contains the DNA binding domain (DBD), and exhibits two zinc finger motif structures conformationally and finished with a carboxy terminus end (CTE).\(^9, 10\) The DBD is the region responsible for the recognition of specific DNA sequences. This region is also important for receptor dimerization. The D domain is mediated the nuclear localization signal (NLS) by demonstrating a flexible hinge zone feature and interacts with DBD. E domain contains the ligand binding domain (LBD) and serves as a formation site of secondary nuclear localization signal (NLS). Activation function-2 (AF-2) is the main domain structure responsible for ligand-dependent activation of ER. F domain has not been found to have a complex regulatory role.\(^5, 6, 9\)

![Figure 1. Human ERα and ERβ nuclear receptors.\(^6\)](image)

**The Molecular Signaling Mechanism of ER**

1. **Genomic signaling mechanism:**

Estrogen affects the physiology of many target tissues. This long-lasting effect of estrogen is mediated by ER’s. ER’s perform their roles as transcription factors that bind to DNA. ER’s facilitate gene expression either directly (Classical) or indirect (Non-classical) binding to DNA through their expression profiles. These pathways are also referred as genomic or nuclear-initiated steroid signaling (NISS).\(^11\)

1.1. **Classic Genomic Mechanism:** Non-ligand-bound ER’s are normally localized in the nucleus of the cell, in the form of monomers or dimers with the DNA molecule.\(^12\) In the absence of the estrogen molecule, estrogen receptors are inactive and bound with heat shock proteins (HSP). However, when the ligand binds to the receptor, in other words when the estrogen molecule enters the cell and is transferred to the nucleus, the estrogen binds to its receptor, and the ligand binding domain (LBD) of the receptor is conformationally changed and receptor is dimerized.\(^13\) Following this event, the estrogen receptor complex binds to estrogen response elements (ERE’s), which are regions localized near the genes controlled by the estrogen and ending in specific sequences. After they bind to ERE’s, transcriptional co-activator or corepressor complexes are incorporated into the complex by activation of the estrogen receptor complex. These complexes alter the transcriptional activity of ER by interacting with the basal transcription machinery and also forming local chromatin structure modifications.\(^7\)

In this way, many genes near the ER complex begin to be
activated. Active genes mediate the production of mRNA molecules and the synthesis of specific proteins. Synthesized proteins affect cell behavior in different ways, depending on cell type (Fig. 3).\[9, 14\]

Steroid hormone receptors (SHR) act as hormone dependent nuclear transcription factors. Following binding of hormone to receptor, the receptor is separated from heat shock proteins and the receptor translocates to the nucleus. In the nucleus, the receptor is dimerized, then bind to sequences called HRE's on the DNA, and a number of co-regulatory proteins are activated to facilitate gene transcription. If this process is summarized: 1) Binding of hormone, 2) nuclear translocation, 3) receptor dimerization, 4) binding to DNA, 5) incorporation of coregulators to the complex, 6) transcription, 7) proteosomal degradation (Fig. 2).[15]

1.2. Non-classical genomic mechanism:

ER's can also influence gene expression by binding directly to DNA, in addition to their interaction with transcription factors. This function is realized through some factors, such as the Fos/Jun active protein complex (AP-1), cyclic AMP response elements (CRE's) and the Sp1 region, or via molecular mechanisms triggered by estrogen-regulated cyclin D1 and insulin-like growth factor 1 receptor (IGFR1) (Fig. 3).[7] Estrogen downregulates genes with anti-proliferative activity or pro-apoptotic activity, while upregulating genes that regulates cell proliferation and cellular vitality with both classical and non-classical pathways. As a result, cell growth is stimulated while apoptosis is suppressed.[16] The upregulation process is mediated by AF-1 (A/B domain) and AF-2 (E domain) complexes which are two transactivation domains mentioned earlier. While AF-1 activity is regulated by the hormone-independent phosphorylation process, AF-2 activity is regulated as hormone-dependent, as it is located at ligand binding domain.[17]

Estrogen bound ER can induce gene transcription directly or indirectly only AF-1 and AF-2 are active. While each domain may be dominant depending on the cellular environment or type of promoter, there is usually a synergistic interaction between these two effects.[7] When ligand is binded to ER, AF-2 structure formed because of the conformational change occurred in ER, and thus a binding surface forms. This surface is also used for further regulation mechanisms of the co-regulatory proteins. Following the conformational change of ER, the co-regulatory proteins are involved in the complex depending on the specific ERE sequences to which ER binds and also ligand.[18]

Due to the formation of a large complex which is formed as a result of binding of the co-activators, the histone acetyl transferase (HAT) enzyme, which causes chromatin decondensation, is introduced and induces transcriptional activity. However, when the histone deacetylase (HDAC) enzyme is activated in the promoter region, transcription is suppressed due to chromatin condensation.[7]

Unlike AF-2, AF-1 is not ligand-dependent. AF-2 is activated by sequential phosphorylation of serine residues by induction of kinase pathways triggered by growth factor receptors.[7] It has been reported that epidermal growth factor (EGFR), insulin-like growth factor (IGFR) and human
epidermal growth factor receptor type-2 (HER-2) play a role in this mechanism. The mechanism of action of estrogen on AF-1 is still poorly understood.

2. Non-genomic mechanism:

The effect of the membrane-initiated steroid signaling pathway (MIS) is a non-genomic mechanism that takes place very quickly at the rate of seconds/minute. This molecular pathway is functioned through membrane-associated ER's. In particular, ER populations that are located at caveolar rafts (in the platelets) and other domains of the plasma membrane mediates this effect. ER has been in interaction with caveolin-1 and a wide variety of proximal signal molecules. These signaling molecules include G proteins, Src, Ras and B-Raf. Activation of the ER's cause signal cascades involved in G protein activation to engage. G protein activation through ER causes the stimulation of phospholipase C (PLC), protein kinase C (PKC), ERK, phosphotidylinositol 3 kinase and nitric oxide synthase (NOS) (Fig. 3). With the induction of these kinases; ER and its co-regulators return to their phosphorylated state, providing activation of growth factors and cytoplasmic kinases. This demonstrates a strong synergism between non-genomic and genomic mechanisms. Non-genomic activity is highly regulated by ligand-dependent fashion and regulated via co-regulatory proteins, and interacts with pathways involved in signal transduction in the cell.

Ubiquitin Proteasome Pathway

In eukaryotic cells, the ubiquitin proteasome pathway is the main system leading to the selective degradation of regulatory proteins, particularly those with short half-life. These include the nuclear receptor family. The ubiquitin-proteasome pathway has functions in many cellular processes. These functions include: regulation of cell cycle, signal transduction, differentiation, antigen processing, tumor suppressor degradation and directing some proteins to cellular localization. Moreover, this pathway selectively degrades various transcription factors such as NF-κB, STAT-1 and fos/jun with short half lives.

In the presence of ligand, ER alpha rapidly undergoes turnover with the ubiquitin proteasome pathway and exhibits a property of a protein with a short half-life. Progesterone receptors (PR) and glucocorticoid receptors (GR) have longer half-lives, approaching 20-25 h, independent of ligand presence.

In protein-mediated protein degradation, ubiquitin (8.6 kDa), which has a high protection property, is covalently bound to the target protein’s lysine residue, and then poly-ubiquitin chain is covalently attached to the complex for marking of this protein for degradation and then substrate protein is degraded. Ubiquitinated proteins are recognized and degraded by multi subunit protease complexes. The best example of this is the 26S proteasome.

The protein ubiquitination pathway contains three different enzyme systems. E1-ubiquitin activating enzyme (E1-UBA), ubiquitin conjugating enzyme (E2-UBC) and E3-ubiquitin protein ligase (E3-UPL). UBA activates ubiquitin in ATP dependent manner. Through the three enzymatic reactions mentioned, ubiquitin residues are linked to the substrate protein which are targeted to 26S proteasomes.

If the mechanism of pathway is examined it has been observed that a thioester bond between the carboxyl end of the glycine residue of the active ubiquitin and the cysteine residue of UBA is formed. Then ubiquitin is transferred from E1 to one of the enzymes in the E2 class and the high energy of the thioester bond is preserved.

In some cases, ubiquitin can be directly transferred from E2 to the target protein with the formation of a peptide bond between the target protein’s lysine residues e-amino and the carboxy terminal of ubiquitin. Another alternative pathway is the transfer of ubiquitin from one of the UBC (E2) to the target protein via E3-ubiquitin protein ligase.

Ubiquitin pathway is regulated by the combination of biologically specific UBCs and E3 proteins. It is now known that there are more than 30 UBCs and 25 E3 proteins. In addition, many components of the ubiquitin proteasome pathway have important functions. The most well-known class of molecular elements that mediate these functions is a protein family interacting with nuclear receptors (Nuclear receptor interacting protein superfamily). In this family: SUG1/TRIP1, RS5P/RPF1, EA-AP and UBC9 have been reported to have important roles on the transcriptional activity of ER alpha. It was previously observed in the studies that ubiquitin conjugating enzyme (UBC-9), E6-related protein, E3-ubiquitin protein ligase and RPF1/RS5P are influencing the transactivation functions of receptors by interacting with the nuclear hormone receptor family.

Basal and Ligand-induced ER Alpha Degeneration

Ligand independent basal turnover; In the absence of estrogen, the ER alpha is ubiquitinated and then degraded by ubiquitin proteasome pathway. Ligand independent ER alpha degradation is realized through dynamic interaction between heat shock proteins, co-chaperone and Hsc 70 interacting protein (CHIP) and carboxy terminal of the ubiquitin ligase.

On the other hand in the ligand dependent degradation; Hormone is bound to the receptor in the presence of estradiol and is targeted for degradation. This degradation is mediated through a transcription-coupled pathway that requires novel protein synthesis (blocked by the protein synthesis inhibitor cyclohexamide). However, for ligand independent degradation of ER alpha, neither transcription activity nor new protein synthesis is needed. As an example for that process, chain of events that occur when ER alpha binds to selective estrogen down regulators (SERDs)
Receptor Concentration and Estrogen Response

One of the main parameters in limiting the estrogen response is the regulation of the ER concentration in the target cells. Steroid hormone activity is dependent on the cellular receptor concentration. Physiologic ER has been shown to limit transcriptional activity of estrogen and reduce cellular capacity for estrogen response. Events related to receptor synthesis and degradation are regulated through transcriptional, posttranscriptional, and posttranslational mechanisms.

These steroid receptor levels in the cells vary depending on the physiological status. The main primer for ER stability is the endocrine regulator, the ligand itself (eg, estradiol). Researchers tried to explain how estrogen controls ER levels with several mechanisms. It has been determined that half life of ER is 5 days in the absence of estradiol, and on the contrary half life of ER is only 3-4 hours in the presence of estradiol. ER protein has a short half-life in the presence of ligand. The estrogen activity and the steady state protein level of ER are reduced by about 60%. In addition to this, it has also been reported that ER protein decline after 1 hour of estrogen administration is independent of protein synthesis and transcription. Studies have shown that estrogen induces a decrease in ER protein levels in PR1 cells in a manner similar to the function of estrogen in MCF-7 cells. In a study conducted on PR1 cell culture, it was determined that after 1-2 hours of treatment of the cell line with estrogen, ER protein levels decreased without causing a change in mRNA level. In this way, rapid loss of ER protein has been shown to regulate ER protein independently of transcription, without altering the levels of ER-mRNA.

Although certain ligands stimulate receptor degradation, it is not clear at what concentration of ER these ligands interact with ER. These results suggest that in the ligand-specific regulation of ER alpha proteolysis, proteolysis is induced at only a certain level of cellular receptor concentration, thereby increasing the efficiency of receptor degradation at this concentration.

ER Alpha Agonists and Antagonists

Intervention with estrogen receptors is accomplished using certain therapeutic agents. These therapeutic agents includes selective estrogen receptor modulators (SERMs), selective estrogen down regulators (SERDs), luteinizing hormone releasing hormone inhibitors (LHRH) and aromatase inhibitors (AI). In premenopausal women, LHRH agonists are working to block estrogen production in ovaries. Tamoxifen, a SERM class used in the treatment of premenopausal women, partially blocks estrogen receptors. The androgen inhibitors used in postmenopausal women inhibit the aromatase enzyme and thus the conversion of androgens to estrogens is inhibited. SERMs and SERDs are also used in postmenopausal women, indicating that their effects on ER-mediated cell replication are strongly linked to estrogen receptors and their degradation.

Selective Estrogen Receptor Modulator (SERM) Tamoxifen

The most important example of the SERM class is the tamoxifen prodrug whose anticancer properties are currently investigated. If mechanism of actions of estriodiol, one of the estrogen molecules, and tamoxifen are compared; it has been observed that in case of estrogen stimulation both ER's are dimerized and AF-1 and AF-2 domain complexes on ER alpha is activated. These receptor dimers move toward the estrogen response elements (ERE) in the nucleus. Subsequently, the AF-1 and AF-2 regions are associated with co-activators and tumor growth is activated through estradiol. In the presence of tamoxifen, the receptor is still dimerized, although AF-1 activation occurs, AF-2 is blocked. The receptor dimer moves to ERE, which binds only to AF-1 co-activators due to AF-2 blockage, resulting in partial inactivation of the transcription process. In the presence of tamoxifen, AF-1 is active because it is not ligand-dependent, whereas AF-2 activity is blocked because it is hormone dependent. As a result, while transcription of genes linked to AF-1 occurs, transcription of AF-2 dependent genes do not occur and thus some of the genes involved in cell proliferation are not stimulated. Intervention to the estrogen receptors through aforementioned meth-
ods partially blocks the effect of estrogen and suppresses the proliferation of breast cells (Fig. 4). [14]
In the presence of estradiol, ER dimerizes and both AF-1 and AF-2 regions become active. The receptor dimer passes through nucleus and binds to estrogen response elements (ERE). AF-1 and AF-2 activate co-activators and estradiol activate tumor growth. When tamoxifen is added, the receptor dimerized, while AF-1 is activated, AF-2 is blocked. The receptor dimer is moving to the ERE, only AF-1 coactivators are activated because of AF-2 blockage which in turn result in partially inactivated transcription (Fig. 4). [44]
Tamoxifen has both estrogenic and antiestrogenic activity depending to the target organ. Thus tamoxifen, a selective estrogen receptor modulator (SERM), is classified as a partial antagonist. Besides the clinical benefits of tamoxifen, it has some side effects and can sometimes cause serious toxicities.Tamoxifen exhibits antiestrogenic effects in the breast and brain tissue, thus reducing breast cancer development and recurrence. Besides, tamoxifen shows estrogenic effect in bone, liver, uterus and leads lipid proliferation and bone density increase. Through this mechanism of action, estrogen can lead to an increased risk of both thromboembolic diseases and uterine cancer. [45]

Selective Estrogen Down Regulator Fulvestrant
Fulvestrant, a member of the selective estrogen downregulators (SERD) class, binds to the estrogen receptor and stops the estrogen signal in the cell. Fulvestrant does not have agonistic activity as opposed to tamoxifen from SERMs. Therefore, Fulvestrant maintains bone density in patients, does not increase blood clotting and does not increase the risk of endometrial cancer. Fulvestrant is a useful form of treatment for estrogen receptor-positive postmenopausal patients who develop tamoxifen resistance. For use in premenopausal women, the adjuvant set should be identified for efficacy of treatment. [46]

The ER binding affinity of Fulvestrant is significantly higher

Figure 4. Comparison of the effect of tamoxifen with estradiol.[43]
than that of SERMs. Fulvestrant, a natural ER antagonist, preventing ER dimerization by binding estrogen receptor, leading to a decrease in cellular ER expression and rapid proteosomal degradation of ER. The ER antagonist, fulvestrant, blocks estrogen activation because of its property of being 7-alpha alkylsulfinyl analog of 17-beta-estradiol. Tamoxifen is separated from other SERM class members, such as raloxifene, due to non-steroidal difference in chemical structure. Fulvestrant competitively inhibits the binding of estradiol to ER. The competitive inhibitory effect is demonstrated with 89% binding affinity for ER with respect estradiol. This ratio is significantly higher than the binding affinity of tamoxifenin to ER.

Fulvestrant-ER complex demonstrates its effect through impairing receptor dimerization and energy dependent nucleocytoplasmic shuttle structure, thereby blocking nuclear localization of the receptor. In addition, the fulvestrant-ER complex is transcriptionally inactive because of damage in AF1 and AF2 complexes, even if they are translocated to the nucleus. In conclusion, the lack of stability of the fulvestrant-ER complex leads to an accelerated ER protein degradation compared to estradiol and tamoxifen dependent ER degradation.

Down regulation in cellular ER protein occurs without a decrease in ER mRNA level. Thus, binding of fulvestrant accelerates the degradation by blocking ER protein, leading to the inhibition of ER dependent estrogen signaling.

**Luteinizing Hormone Releasing Hormone (LHRH) Agonists**

Another endocrine therapy method in premenopausal women is LHRH administration. This treatment protocol can temporarily suppress ovarian estrogen. This is accomplished by desensitizing the hypothalamus/pituitary/ovarian axis. This therapy method is highly recommended compared to applications such as radiotherapy or oophorectomy. LHRH agonists reliably and reversibly block the estrogen synthesis in the ovary. In premenopausal advanced breast cancer patients, the combined use of LHRH agonists and tamoxifen has been reported to yield better results than administration of LHRH agonists alone.

**Aromatase inhibitors (AIs):** Antiaromatase agents inhibit the cytochrome P450 enzyme which is responsible for the final step of estrogen biosynthesis and which is one of the components of the aromatase enzyme complex. These drugs are classified according to their formation. Third generation agents are used for breast cancer treatment. This group of drugs is divided as type I and II in it. Exemestane can be provided as an example of type I inhibitors. This class of inhibitors is analogous to androgens in terms of steroidal structure and inhibits the enzyme by irreversibly blocking it from the substrate binding site. Nastrazol and letrozole among Type II inhibitors are non-steroidal and their effects are reversible. Als ceases estrogen biosynthesis originated from breast and adipose tissue-derived androgens. This biosynthesis is catalyzed using the aromatase enzyme. These inhibitors inhibit the aromatase enzyme. It has been reported that use of anastrazole, letrozole and exemestane in advanced postmenopausal breast cancer patients was found to be superior to tamoxifen therapy. The successive use of AI and tamoxifen has been reported to reduce side effects of treatment. The use of AI has been shown to be unsuitable for treatment of pot menapousal patient with intact hypothalamus/pituitary/ovarian axis. Among these patients, these drugs can be safely administrated to patient whose hypothalamus/pituitary/ovarian axis is repressed. Joint administration of tamoxifen and LHRH agonists together with chemotherapy are preferred, particularly in the treatment of premenopausal patients.

**Conclusion**

In the previously conducted studies it has been shown that E2 gene expression regulation is multiformal process realized through genomic and non-genomic signaling mechanisms. Target gene response can be affected by many different stimuli. Different combinations of transcription factors binding to specific gene promoters, cellular localizations of ERs, levels of multiple co-regulatory proteins, components involved in signal transduction and natural extracellular inducers, can be listed among these stimuli. These variables or stimuli have cell type-specific functions. Thus, E2 can cause different gene responses in different types of target cells by using different signaling pathways, depending on the physiological state of cell and cell.

The cell-specific environment (eg, differentiation, ER level, ER co-expression, etc.) can affect the rapid signal response of E2 in the cell membrane and subsequent nuclear transcription. This results in the formation of different signaling pathways, formation of different gene expression profiles for same hormone response, and different biological consequences. Future targets includes definition of the effect of each ER in the intracellular pool for clarification of the net role of ER beta, and elucidation of the molecular mechanisms underlying the possible interactions between ERs and other nuclear receptors. Through investigation of complex control mechanism of ER in a more detailed way, important players in this molecular process can be identified and target candidates planned to be used in therapeutic steps in the treatment of hormone sensitive diseases such as cancer can be determined.
Disclosures

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