REVIEW
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Breast cancer and the molecular mechanism of estrogen signaling

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Abstract

Cancer is a complex pathology that occurs due to the uncontrolled proliferation and growth of cells in any organ or tissue of the body. Breast cancer is the most frequently diagnosed cancer among women worldwide and is the second leading cause of cancer-related deaths. Breast cancer is a pathology that exhibits heterogeneity in which genetic and environmental risk factors play a role. Although many treatment approaches have been developed for breast cancer today, the frequency of the number of patients diagnosed with breast cancer and lost their lives due to this reason is increasing in the world. The most significant limitation to the success of the treatment approaches developing drug resistance in breast cancer cells, and the disease relapses after a certain period and exhibits a more aggressive profile. Therefore, understanding the molecular biology of breast cancer is essential for developing potent therapeutic approaches. It is known that the development of breast cancer is related to changes in direct and indirect signaling mechanisms mediated by estrogen and estrogen receptor. These signaling mechanisms exhibit highly complex interaction patterns. This review summarizes the pathology of breast cancer, estrogenic compounds, estrogen receptors, genomic and non-genomic molecular signaling mechanisms mediated by estrogen and estrogen receptor.

Keywords: Breast Cancer, Estrogen, Estrogen Receptor

INTRODUCTION

Breast Cancer

Cancer is a commonly observed pathology due to the uncontrolled proliferation of cells in any organ or tissue of the body. Today, breast cancer is the most commonly diagnosed cancer type in women worldwide and is the second leading cause of cancer-related deaths (1). Breast cancer has a wide variation in gene expression level and is a complex disease with genetic and clinical heterogeneity. (1). This heterogeneity determines the progression of cancer, treatment success and survival rates of patients (2). Breast cancer refers to a malignant tumor originating from cells in the breast tissue. Breast tissue has 15 to 20 sections called lobes, which are organized into much smaller sections called lobules. Lobes and lobules are connected by thin tubes called ducts (3). While breast cancer is limited in the channel system (ducts) that carries milk during its initial development, tumoral cells are infused into the connective tissue by advancing over the basement membrane, depending on the progression in the carcinogenesis. Breast cancer can spread to different parts of the body through the inclusion of cancerous cells in the blood or lymphatic system. This process is referred to as metastasis. (4). Although breast cancers can originate from different parts of the breast tissue, they often develop over the inner wall of the lobules in the breast tissue. Theoretically, breast cancer tissue with a mass of one gram is estimated to develop on average in eight years (4).

According to GLOBOCAN 2023 data, it is estimated that 1,958,310 new cancer cases will be diagnosed, and 609,820 people will die from cancer in the United States. Also, according to these data, the most commonly diagnosed cancer type is breast

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cancer in females for the estimated new cancer cases, which will account for 31% of cancer cases.(6%), breast (11.6%), and colon (10.2%) cancers, respectively (5). Furthermore, according to 2015 data from the Turkish Republic Ministry of Health, breast cancer ranks first among the top 10 cancer types observed in women in Turkey. According to these data, the incidence of breast cancer is 43.8 per hundred thousand (6). Additionally, the International Agency for Research on Cancer reported that the incidence of breast cancer in women increased by 20% and breast cancer-related deaths by 14% compared to previous estimates (5). Today, it is determined that one out of every four women diagnosed with cancer has breast cancer. Considering the general age distribution of breast cancer in Turkey, it is seen that 44.5% of the women diagnosed with breast cancer are between the ages of 50-69, and 40.4% are between the ages of 25-49.(6).

Breast cancer cells are a type of cancer that can often be observed on X-ray examinations or felt as a lump during physical examination. Although it can be seen primarily in women, it can also be seen in men at low frequency. Early diagnosis is essential for determining whether the lumps felt in the breast are benign or malignant breast tumors and for the success of the treatment to be applied. Although non-cancerous breast tumors are mostly foci of abnormally growing cells, they do not spread beyond the breast tissue and are not life-threatening (7,8). However, it is known that some benign and stable breast lumps can increase the risk of developing breast cancer (9). Therefore, it is crucial for early diagnosis, especially for female individuals, to participate in regular screening programs in proportion to their age (10). The most apparent findings related to breast cancer, except the mass development in the breast tissue, are, swellings and collapse in the breast or armpit, discharge, cupping, pain, enlargement, focal asymmetry, ulceration, inflammation findings, orange peel appearance and deformity in the breast (9,11).

Stages of Breast Cancer

Knowing the stage of breast cancer is essential to understanding how quickly cancer cells can grow and spread. Therefore, cancerous tissues taken from patients are staged according to specific parameters by laboratory studies (12). The stage depends on how similar the cancer cells are to normal cells. Breast cancer is classified from stage 0 (zero) to stage 4 (four). Accordingly, stage-0 is not literally defined as breast cancer. At stage-1, the tumor size is less than 2 cm, and the tumor does not spread to another site. In stage-3, the tumor size can be greater than 5 cm or less than 5 cm and it can also be highly adherent in the axillary glands, attached to the chest muscle wall, or spread to lymph nodes in the neck. In stage-4, a large spread of breast cancer to other tissues and organs is observed (13).

The Importance of Inherited and Acquired DNA Mutations in the Development of Breast Cancer

Alterations in DNA sequence or mutations can cause normal breast cells to turn into cancerous. Some alterations in DNA can be inherited from generation to generation, and these can significantly increase the risk of breast cancer. Although other risk factors related to lifestyle and environmental factors may increase the rate of breast cancer development, how some of these risk factors contribute to the cancerization of normal cells is still not fully understood (14).

Some mutations in cells can be an important risk factor for developing various types of cancer. It is seen that a significant part of the mutations observed in DNA is acquired later. Mutations in some genes that are critical for cells lead to uncontrolled division of cells (14). In particular, alterations in tumor suppressor and proto-oncogene genes often result in cancer development. Tumor suppressor genes are associated with cell division control, repairing of DNA errors or programmed cell death. When these genes lose functionality, cells uncontrolled divide and initiate the cancerous (15). The most well-known genes responsible for susceptibility to breast cancer are breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2). Studies have shown a strong relationship between the germline mutations of BRCA1 and BRCA2 and the development of breast cancer (16). In addition, it has been reported that BRCA1 and BRCA2 mutations increase the risk of ovarian, uterine tubes, and peritoneal cancers, while BRCA2 mutations increase the risk of breast cancer, pancreatic cancer, and melanoma in men (17). These groups are being studied under Hereditary Breast and Ovarian Cancer (HBOC) (18). Both genes are inherited in an autosomal dominant manner and constitute a well-defined example of tumor suppressor genes (18). Genes with carcinogenic properties are called oncogenes and these group genes contribute to cell development, controlling the division cycles and normal proliferation of cells. When a proto-oncogenic gene is mutated or has multiple copies, it can become a carcinogenic gene that can remain continuously active. In this case, cells can divide uncontrollably, leading to cancer (19,20). Some effective proto-oncogenes in breast cancers are Ras, human epidermal growth factor receptor 2 (HER2), and c-Myc genes (21,22).

Types of Breast Cancer

Today, many different types of breast cancer have been described. Among the most common types of breast cancer are ductal carcinoma in situ (DCIS) and invasive carcinoma. Some breast tumors, such as phyllodes and angiosarcomas, are less common. Receptor expression levels are used in molecular typing of breast cancer. Following the biopsy, breast cancer cells are analyzed to determine the expression profiles of proteins called estrogen receptor (ER), progesterone

receptor (PR) and HER2. A detailed set of methodological approaches, such as advanced molecular analyzes and extensive histological staining, are used to define the stage of tumor cells. Also, the tumor stage and specific protein expression levels help to decide treatment options (23).

In Situ and Invasive Breast Cancers:

The type of breast cancer can provide information about whether cancer has spread. If cancer originates from the mammary gland is called lobular carcinoma; otherwise, it is named ductal carcinoma. These two groups are divided into two subgroups depending on whether the cancer is still inside or outside the mammary gland or milk duct. Cancer inside the mammary gland is called lobular carcinoma in situ (LCIS) and the outside mammary gland is called invasive lobular carcinoma. The terms invasive and infiltrative mean that cancer cells have spread beyond the ducts of the mammary gland (24).

Ductal Carcinoma in Situ (DCIS):

DCIS; Intraductal carcinoma is a non-invasive or pre-invasive type of breast cancer. One in five newly diagnosed breast cancers determines to be DCIS (25). It is also referred to as intraductal carcinoma or stage 0 breast cancer. DCIS means that the duct cells have transformed into cancer cells but have not spread to the breast tissue near the ducts. In addition, since there is no spread to the breast tissue in DCIS, metastasis is not observed (26).

Invasive Breast Cancer:

Breast cancers that have spread to the surrounding breast tissue are known as invasive breast cancer. It constitutes the majority of diagnosed breast cancers. Invasive breast cancer divided into subclasses. The two most well-known classes are invasive ductal carcinoma and invasive lobular carcinoma. Invasive ductal carcinoma accounts for approximately 70-80% of all breast cancers. Inflammatory breast cancer and triple-negative breast cancer are also types of invasive breast cancer (27).

Invasive (Infiltrative) Ductal Carcinoma (IDC):

IDC is the most common type of breast cancer. About 8 out of 10 invasive breast cancers are diagnosed as invasive (or infiltrative) ductal carcinoma. IDC begins in the cells surrounding the milk duct in the breast and grows from there through the duct wall to nearby breast tissues. In this case, it can metastasize to other body parts through the lymphatic system and blood circulation (28).

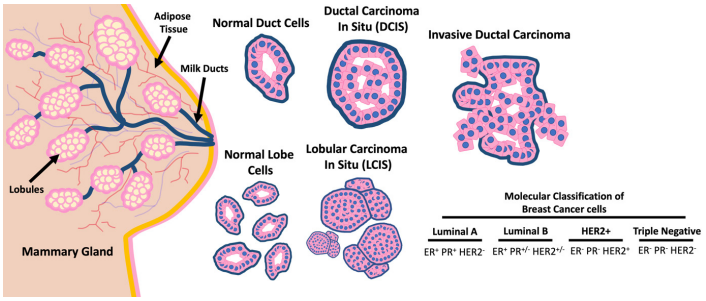

Figure 1: Schematic representation of types of breast cancers.

Invasive Lobular Carcinoma (ILC):

1 in 10 invasive breast cancers is diagnosed with ILC. ILC can metastasize to other body parts, such as IDC, starting in the milk-producing glands (lobules). Compared with other types of invasive carcinoma, ILC could be present in both breasts in 1 out of 5 women (29).

Less Common Types of Invasive Breast Cancer:

Less common invasive breast cancers are also known as special types of invasive breast cancers. Some special types of breast cancers are classified into invasive carcinoma subtypes. These types of breast carcinomas are less common than other types of breast cancer, and each usually accounts for less than 5% of all types of breast cancer (23).

Triple Negative Breast Cancers (TNBC):

Unlike other types of invasive breast cancer, TNBC spreads much faster and has limited treatment options. It also has a worse prognosis compared to other breast cancer types. TNBC is classified as a type of breast cancer in which cancer cells do not have ER or PR. Also, it doesn't express three receptor proteins, including ER, PR, and HER2. TNBC is an aggressive invasive breast cancer that accounts for 10-15% of all diagnosed breast cancers. Also, the treatment options are more limited than in other breast cancer. It has a low treatment success and high recurrence rates. Moreover, it has several subtypes, including Luminal A (ER^+, PR^+, $HER2^-$, Ki-67^{low}), Luminal B (ER^+, PR^{low} or $^-$, $HER2^-$ or $^+$, Ki-67^{high}), $HER2^+$ (ER^-, PR^-, $HER2^{high/overexpressed}$) and Basal-like ($ER^-$, PR^-, $HER2^+$). While various approaches can be offered with hormonal intervention for treating breast cancer subtypes that are positive for one or more receptors (ER, PR, or HER2), hormone-focused therapies cannot be utilized in TNBC (30). Therefore, new treatment approaches for TNBC are under intense research.

Estrogenic Compounds

Estrogens are classified into two main groups as natural and synthetic estrogenic compounds (31). Natural estrogens are classified into three subgroups ovarian steroids, mycoestrogens, and phytoestrogens. PPhytoestrogens have weaker biochemical activity than estrogens produced in the endogenously synthesized estrogens. They are abundantly

found in foods such as soybeans, garlic, parsley, cereals, carrots, potatoes, cherries, apples, and coffee, which are frequently consumed in the daily diet. In addition, phytoestrogens are classified into two groups phenolics (lignans, stilbenes, flavonoids, isoflavonoids) and non-phenolics (terpenoids, saponins). Synthetic estrogens are divided into two groups: birth control pills, estrogen drugs such as diethylstilbestrol, cimetidine, and environmental estrogens, known as lxenoestrogens, which are long-term and persistent organic pollutants and difficult to recycle in nature (32).

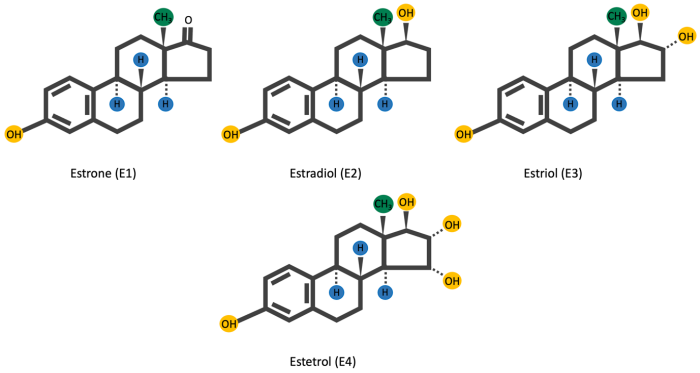

Figure 2: Natural estrogenic compounds. Representation of the chemical structures of Estrone (E1), Estradiol (E2), Estriol (E3) and Estetrol (E4) compounds.

E1 (Estrone), E2 (17β-Estradiol), E3 (Estriol), and E4 (Estetrol), which are especially important for female physiology, are among the natural steroid estrogens (Figure 2). In addition to the adrenal gland, E1, produced in the ovaries, placenta, testicles, and adipose tissue, is the primary estrogen in men, and women who have gone through menopause. E1 is secreted less than other forms of estrogen and is weakly effective (33). E2, dominant in the reproductive period, is secreted from the ovarian follicles and is produced in the ovaries in premenopausal women and the testicles in men. E2, the most potent and abundant estrogen in premenopausal women, is 12 times more active than E1 and 80 times more active than E3 (34). E2 levels in postmenopausal women are usually below 20 pg/ml. During the ovulation cycle, serum concentrations of E2 range from 30 pg/ml in the early follicular phase, 150-350 pg/ml in the preovulatory stage, and 100-210 pg/ml in the luteal phase. During pregnancy, E2 levels increase 100 times (35). E3 is produced in the placenta in women, while it is in the adrenal glands in men. In non-pregnant women, circulating E3 serum concentrations are very low (approximately 10 pg/ml) as E3 is rapidly cleared from circulation. During pregnancy, the level of E3 reaches from 12 ng/ml to 210 ng/ml (35,36). E4 is an estrogenic steroid molecule synthesized only by the fetal liver during pregnancy in women and reaches the maternal circulation via the placenta. It is only synthesized during pregnancy, while other forms of estrogen can be continuously observed in circulation (37).

Breast Cancer Associated Receptors

Breast cancer is a complex pathology that exhibits a high level of heterogeneity and has different genetic alterations. After the 2000s, breast cancers have been begun to be categorized according to their gene expression profiles and histopathological typing. It is classified into four subtypes, mainly Luminal A, Luminal B, HER2 overexpression, and triple negative, also known as basal-like. ER and PR are positive in Luminal A and Luminal B subtypes. There are also differences between subtypes regarding clinical behavior, survival rates, and response to treatment. The evaluation of ER, PR, and HER2 expression levels is used as prognostic and predictive factors. Tumor cells are classified as hormone receptor-positive or negative, depending on their receptor expression profile. Knowing the expression status of the hormone receptor is an essential factor in selecting the treatment strategy to be applied (38). In the follow-up of this section, basic information about the ER protein, which is known to be closely associated with breast cancer, and signaling pathways controlled by the ER will be shared.

Estrogen Receptor

ER is a steroid/nuclear receptor superfamily member. It is a specialized transcription factor stimulated by estrogens and regulates a series of complex signaling processes within the cell (39). Physiologically, ERs are involved in women's menstrual cycle, during pregnancy and lactation, as well as the functioning of the cardiovascular system, nervous system, musculoskeletal and immune system, and the regulation of the responses of these systems to stimuli (40,41).

The ER protein is encoded by the estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) genes located on separate chromosomes in the human genome. These genes mediate the formation of two isoforms, ER alpha (ERα) and ER beta (ERβ) proteins. The ESR1 gene is located at position 6q24-27, and the ESR2 gene is at 14q22-24 (42). ERα and ERβ are involved in the regulation of many complex physiological processes in humans. The full-length ERα isoform consists of a polypeptide chain of 595 amino acids and has a molecular weight of approximately 67kDa. The shorter isoforms of ERα, which have 36kDa and 46kDa molecular weights, are synthesized through alternative splicing or start codon. Some of these short isoforms lack the activation function-1 (AF-1) region located in the N-terminal domain (NTD) required for transcriptional activation. Although these isoforms are not incapable of transcriptional activation, they can heterodimerize with full-length ERα and block transcriptional activity (43). ERβ is 530 amino acids long and has a molecular weight of 59kDa (Figure 3). Both receptor forms regulate

the transcriptional program of target genes by interacting with specialized regulatory DNA sequences in the nucleus. The main difference observed between full-length ERβ and shorter ERβ isoforms is in the C-terminal ligand binding domain (LBD). Therefore, ERβ isoforms lacking transcriptional activity are capable of suppressing ERα signaling by dimerizing with ERα (44). In addition, ERα and ERβ receptor forms have subtypes with different functionality. Some of these types are ERα36 and ERα46, which lack the AF-1 region, and ERβ2/cx and ERβ5, which lack the F region and show differences in the length of the LBD (45).

ERα and ERβ have different expression patterns in tissues. ERα is mainly expressed in the mammary gland, uterus, thecal cells of the ovary, bone, and male reproductive organs such as the testis and epididymis, liver, and adipose tissue (46). ERβ is expressed in prostate epithelium, bladder, ovarian granulosa cells, colon tissue, immune system cells, and adipose tissues. Differences are observed in the expression foci of both subtypes of ER, it is known that both ERs are expressed in the tissues of the cardiovascular and nervous systems. Although the effects of ERs on the mammary gland and uterus are more widely known, they have several critical roles, including maintaining the cardiovascular system, homeostasis of skeletal muscle, and regulating metabolic flow (47). Besides, ERβ actively regulates the central nervous system and immune system responses. In contrast, it has an antagonistic biological effect against cell hyperproliferation supported by ERα in tissues such as the breast and uterus. Also, ERα has active roles in regulation of metabolism and maintaining skeletal system homeostasis as well as on the mammary gland and uterus (39).

Like other nuclear hormone receptors, ERs have structurally specialized functional domains and segments organized within these domains to perform individual functions. These domains are NTD, DNA binding domain (DBD), and LBD. Moreover, the AF-1 and activation function-2 (AF-2) regions located in NTD and LBD, respectively, are responsible for regulating the transcriptional activity of the ER. The AF-1 region is hormone-independent, while the AF-2 region functions in the presence of hormones. ER protein is divided into 5 main structural divisions: A/B domain, C domain, D domain, E domain, and F domain. Basically, the difference between the two forms of receptors is that ERβ has a shorter NTD than ERα. The A/B region is represented by a zinc finger-containing NTD domain that participates in the transactivation of gene transcription and mediates binding to the target sequences (48).

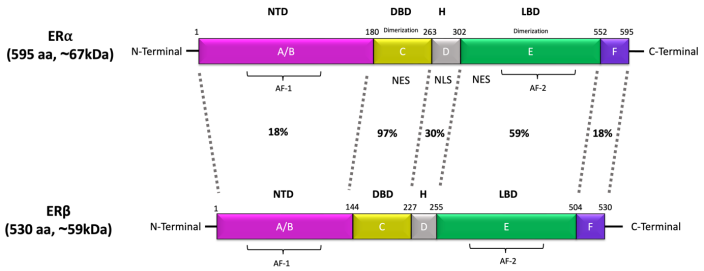

Figure 3: The protein organization of ERα and ERα. Representation of protein structure, functional domains and locations, homology ratios and protein structures of ERα and ERα.

Region C corresponds to the DBD of the ER, which helps dimerize and interact with specialized sequences in the chromatin structure. The DBD domain interacts with the 5′-AGGTCAnnnTGACCT-3′ estrogen response element (ERE), usually a palindromic hexanucleotide consensus motif on DNA. The DBD domain of ERα and ERβ isoforms may share the same DNA response elements (49). Further studies have determined that EREs also regulate the participation of coregulators in the ER transcriptional module (50). Furthermore, these elements share a high degree of sequence similarity, while it is known that the intrinsic sequence composition of EREs can regulate the receptor's binding affinity to DNA (51). The P-box, located in the ER's protein structure, interacts effectively with EREs in the interaction of ERs with nucleic acids. The D-box mediates the formation of an interface for dimerization. Moreover, D domain connects the C and E domains, is located between the DBD and LBD, and forms a flexible hinge region that allows interaction with molecular chaperone proteins. In addition, this region contains the signal sequence required for nuclear localization (52,53).

LBD is located in the E domain of the ER protein and consists of 12 helix structures. LBD functions as a ligand binding and dimerization interface. Also, it contains a hormone-binding pocket. When the LBDs of ERα and ERβ are compared in terms of structural similarity, it is seen that the homology between the LBD domains of both proteins does not exceed 55%. Although the homology ratio between the LBDs of the nuclear receptor superfamily members is low, there is a remarkable similarity in their 3-D structural organization. This structural feature is likely the product of adaptive plasticity gained in evolution to make the interaction of each receptor protein with the corresponding steroid hormone specific. The LBD of ERα contains the hormone-binding domain, the interface that mediates homo- and heterodimerization, and interaction surfaces on which the interactions of activators and inhibitors are coordinated. The C-terminal F domain downstream of LBD regulates the ligand-specific gene expression. Besides, it has been reported to be effective in receptor dimerization (52,54). In addition, the E/F region contains binding sites for the interaction of accessory proteins and cofactors (55).

Depending on the composition of various post-translational modifications, the activity and interactive protein dynamics of ERα and ERβ change. Numerous modifications for ERα and ERβ have been reported in the literature. These modifications include phosphorylation, methylation, acetylation, SUMOylation, ubiquitination, acetylation, palmitoylation, and glycosylation (45). Phosphorylation of ERα at positions Y52, S102, S104/106, and S118 through kinase proteins can increase or decrease its transactivation ability. For example, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and E2 stimulation-mediated mitogen-activated protein kinase (MAPK) induced S118 phosphorylation of ER result in the transcriptional activation. In contrast, reactive oxygen species (ROS)-mediated MAPK stimulation may cause a decrement in receptor expression levels. Phosphorylation at the S80 position for ERβ has been reported to promote ERβ degradation. The ubiquitination at positions K302 and K303 has been shown to target ERα for proteasomal degradation (45). Post-translational modifications of different isoforms and subtypes of ERs and tight interaction dynamics with their co-regulatory proteins mediate ER-mediated tissue-specific responses. Therefore, the mechanism of ER-mediated signaling is discussed under several sub-headings in the following sections.

ER-mediated Signaling

Nuclear Estrogen Receptors

Genomic Signaling: Estrogens directly pass the plasma membrane and fulfill the specific biological functions through inducing the ERα and ERβ receptors. Depending on the targeted cellular effect and molecular response in estrogen signaling, the estrogen response has been divided into genomic and non-genomic signaling. Genomic signaling encompasses a series of processes, including translocation of the complex formed after the estrogen-ER interaction to the cell nucleus and direct interactions with chromatin-mediated by specific DNA sequences known as ERE. Non-genomic signaling refers to the control of estrogen signaling through other intracellular signaling networks (56). Directly maintained genomic signaling is also known as the classical estrogen signaling pathway. After binding of estrogen to ERα or ERβ in the cytoplasm of the cell, a series of conformational changes occur that induce dimerization of the receptor (57). Subsequently, the activated receptor complex translocates into the cell nucleus. It regulates gene expression processes by selectively binding to ERE sequences in the chromatin structure, 3'-untranslated regions of target genes, or areas adjacent to the promoter region (58).

Non-genomic signaling: ERs can regulate the expression of some genes through interactions with other co-regulatory proteins without directly interacting with DNA. Recent studies suggest that 35% of genes regulated by estrogens do not have a putative ERE sequence in their promoter region (44,56). Regulation of estrogen-mediated expression of these genes is controlled through protein-protein interactions that ERs maintain with protein groups that interact with other transcription factors and response elements. In this, estrogens can activate or suppress the expression of target genes. In addition, non-genomic steroid signaling responses tend to be rapid and sensitive (59,60). Non-genomic signaling includes a series of events, including mobilization of seconary messengers, membrane receptors such as insulin-like growth factor-1 receptor (IGF-1R), epidermal growth factor receptor (EGFR), and interaction with Src, phosphoinositide 3-kinases (PI3K), and protein kinase B (PKB) (61).

One of the most important mediators in the non-genomic signaling process is specificity protein 1 (Sp-1). The presence of ER enhances the interaction of Sp-1 with its targets. Today, many genes related to this mechanism have been characterized. A few of these targets are PR-B, signal transducer and activator of transcription 5 (STAT5), low-density lipoprotein receptor (LDLR), GATA Binding Protein 1 (GATA1), and retinoic acid receptor-1 alpha (RAR-1α) (55, 62, 63). In addition, ERα is capable of interacting with other transcriptional modulators, such as activating transcription factor-2 (ATF-2), c-jun, and activating transcription factor-1/cAMP-responsive element (CRE)-binding protein (ATF-1/CREB) (62). Also, ERα regulates the expression levels of target genes containing activator protein-1 (AP-1) transcription factor interaction domains through protein-protein interactions (64). AP-1 is a transcription factor that regulates essential cellular processes such as cell differentiation, proliferation, and apoptosis (55). The best-known examples of target genes controlled by AP-1 are IGF-1, collagenase, IGF-1R, ovalbumin, and cyclin D1 (56,65). It is known that the two main isomers of ER regulate the expression level of target genes with different patterns. E2, a potent estrogenic molecule, regulates AP-1-dependent transcription through ERα, while ERβ can inhibit this mechanism (66). A well-understood example of this mechanism is the regulation of cyclin D1. Estrogen-stimulated ERβ suppresses cyclin D1 expression, while ERα has an opposite effect on cyclin D1 expression in the presence of both receptors (56,67). This regulation model provides a good example of the mechanisms involved in controlling tissue-specific estrogen-mediated gene expression processes based on the tissue expression profiles of ER isoforms and other transcription factors. Also, other steroid hormones and their receptors regulate gene expression processes by similar mechanisms. Therefore, elucidating the dynamics of receptor isoforms and their interactions with other coregulator proteins is essential in understanding the processes related to hormone-dependent cancers, including breast cancer.

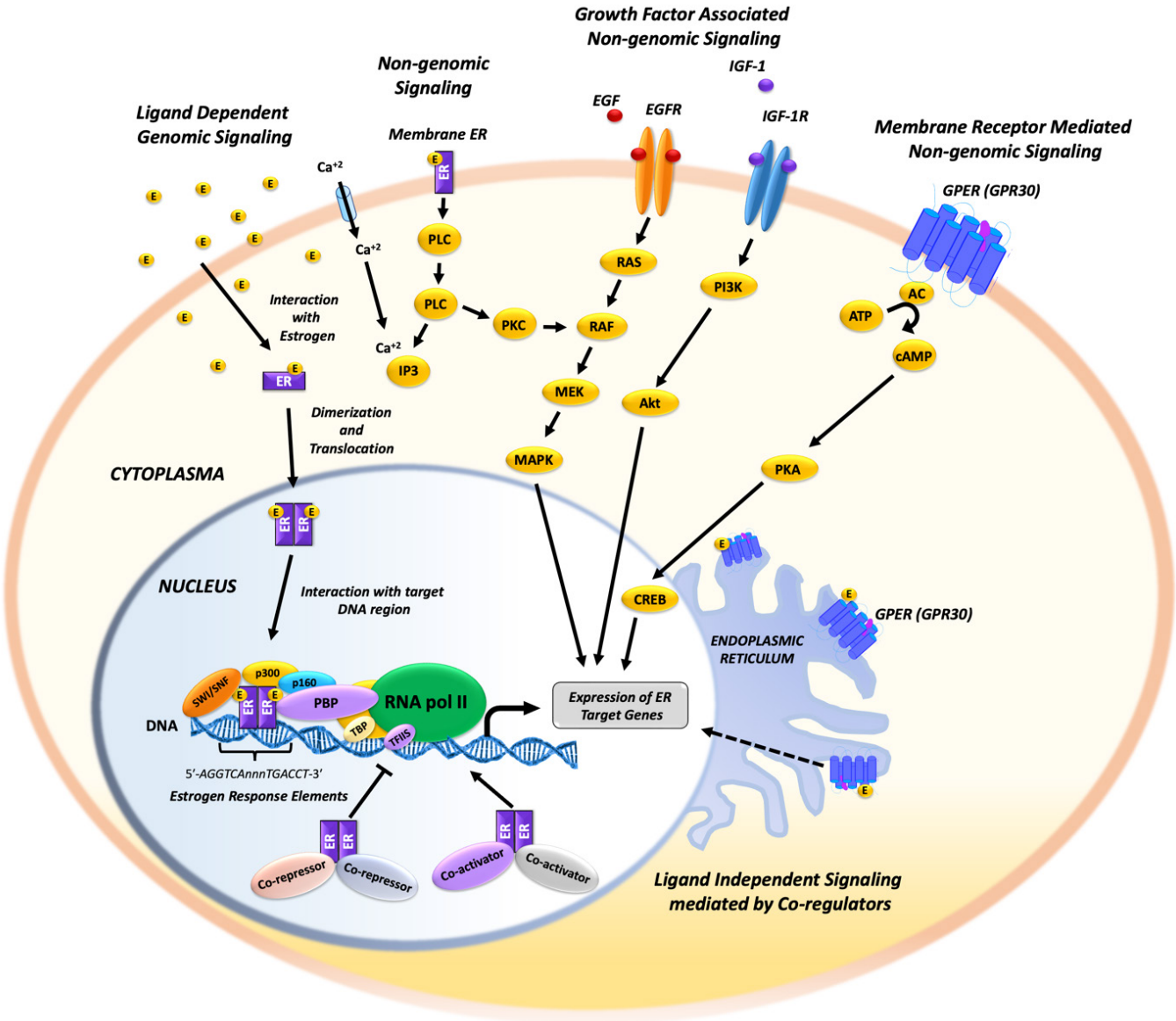

Figure 4: ER-related signaling mechanisms. Demonstration of ER-mediated ligand-dependent genomic signaling, non-genomic signaling, growth factor-related non-genomic signaling and membrane-mediated non-genomic signaling mechanisms in breast cancer.

Non-genomic Signaling Mediated by Membrane Receptors

The activity of the non-genomic signaling mediated by estrogen stimulation is driven by a series of signaling cascades mediated by intracellular levels of secondary messengers such as cyclic adenosine monophosphate (cAMP) and activation of protein kinases (68). The activities of target proteins can be highly coordinated through post-translational modifications such as the phosphorylation of proteins. By this way, many cellular signaling mechanisms, including ERα and ERβ, can be highly coordinated. ERα and ERβ are good molecular targets for kinase proteins. G Protein-Coupled Estrogen Receptor 1 (GPER1), a membrane-bound ER, and some variants of ERα and ERβ have been associated with non-genomic estrogen signaling (69,70). ERα, Erβ, and GPER can function synergistically or antagonistically at the cellular level. These cross-interactions mediate the formation of complex physiological responses to various stimuli (71). Therefore, to improve our understanding of the biology of breast cancer, all signaling cascades regulated by estrogen, ER-mediated direct and cross-interactions-coordinated mechanisms need to be further characterized.

GPER was identified in the late 1990s and is a protein belonging to the G protein-coupled receptor (GPCR) family that possesses seven membrane-spanning domains and is a protein belonging to the G protein-coupled receptor (GPCR) family that possesses seven membrane-spanning domains GPER was identified in the late 1990s. GPER, also known as GPR30, interacts with a heterotrimeric G protein and mediates the regulation of multiple intracellular signal transductions. It is also involved in rapidly activating extracellular signal-regulated protein kinase 1/2 (ERK1/2)

mediated by E2. The ability of GPER to activate adenylate cyclase has been implicated as a mechanism involved in the activation and/or inhibition of ERK1/2. Moreover, GPER activates the PI3K/Akt pathway and responds to E2 by EGFR transactivation. Furthermore, GPER can control the various signals such as c-fos and cyclin D1, connective tissue growth factor, fatty acid synthetase, and vascular endothelial growth function at the gene expression level. These different GPER-mediated signals regulate various physiological processes such as cell proliferation, metabolism, migration, and secretion (73). ERα and ERβ can interact with G proteins, tyrosine kinases, membrane receptors such as IGF-1R and EGFR, and signaling pathway molecules such as Ras, Src, and PI3 kinases, and HER2. These interactions can precisely regulate the expression levels of target genes with MAPK and Akt signal transduction pathways, which are associated with various cellular responses (74).

Ligand Independent ER Signaling

Ligand-independent ER signaling is tightly regulated with regulatory molecules required for phosphorylation, such as kinase cascade components, including protein kinase A (PKA), Protein kinase C (PKC) and MAPK, inflammatory cytokines, cell adhesion molecules, cell cycle regulators, and peptide growth factors; EGF, insulin, IGF-1 and transforming growth factor beta (TGF-β) (75). However, the details of signal transduction mechanisms involving ER proteins in the absence of estrogenic compounds and other receptor agonists are still poorly understood (44). Post-translational modifications regulate the special biochemical functions of numerous proteins. In particular, the phosphorylation of serine, threonine, and tyrosine residues, mediated by various kinase enzymes, plays a vital role in regulating many biochemical functions. Momentary alteration of phosphorylation patterns has an essential role in coordinating specific responses in ligand-independent ER signaling. Furthermore, it has been suggested that estrogen receptor-mediated intracellular signaling is highly regulated through specific modification motifs in the ER protein structure of ERs (44).

The transcriptional activity of the ER-mediated signaling mechanism is regulated by groups of proteins expressed as coregulators. While interaction with coactivators increases the transcriptional activity of ERs, corepressors cause transcriptional repression. Coregulators play a crucial role in many step of the signal transduction process, such as rearranging chromatin structure, transcriptional initiation, RNA chain elongation, mRNA processing, and translation (76). One of the first identified coregulators for ERα is steroid receptor coactivator-1 (SRC-1) (77). Although a limited number of coregulators have been characterized for ERβ, numerous coregulatory proteins have been identified that regulate the activity of ERα today (78). Some of these regulators for ERα are ATP-dependent chromatin remodeling systems such as SRC/p160, histone acetyltransferase, CREB-binding protein (CBP)/p300, SWItch/Sucrose Non-Fermentable (SWI/SNF), and E3 ubiquitin ligase enzymes (78,79). Protein dynamics regulated by diverse protein complexes, including coregulatory proteins, have critical importance in terms of precise control of expression levels of target genes and the formation of tissue-specific responses (79).

Coregulator proteins have specific structural motifs and selectively maintain interactions through these motifs (78). In particular, LxxLL motifs mediate these interactions. The interactions between corepressors and free ER proteins competitively take place with coactivators (79). On the other hand, intracellular levels of coregulators and post-translational modifications such as phosphorylation, methylation, or ubiquitination control the ER-mediated gene expression processes by modulating coregulator dynamics. In this way, ER-mediated signal transduction is indirectly fine-regulated by highly coordinated protein interactions (76,80).

All this information summarized in this review regarding estrogenic signaling in breast cancer cells reveals the complexity of breast cancer biology. The cross-protein interactions, altered chromatin dynamics, and dynamic processes of alternative post-translational modifications occurring in the absence and presence of estrogenic stimulation is the most compelling obstacle to a complete understanding of breast cancer biology. This situation constitutes the biggest obstacle in front of new treatment strategies to be developed. Therefore, there is a great need for extensive further analysis and ongoing studies on discovering new regulation models.

CONCLUSION

Breast cancer biology constitutes an area on which intensive studies continue, especially the signaling mechanisms regulated by breast cancer-related receptors are tried to be understood. In this review, the basic information about breast cancer and the mechanisms related to estrogen and ER-mediated signaling, which are thought to play crucial roles in breast cancer, are summarized at a basic level. To achieve effective treatment success in breast cancer, there is a need for multidisciplinary approaches to clarify the unknowns of breast cancer-related signaling mechanisms and their possible interactions with other cellular signaling networks.

Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Since this study is a review article, ethics committee approval is not required, and the Helsinki Declaration rules were followed to conduct this study.

Author Contribution

Concept: YE, Design: YE, Supervision: YE, Literature search: YE, HKD, Writing: YE, HKD, Critical review: YE, HKD.

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