Chapter

The Role of Estrogen Receptors and Signaling Pathways in Breast Cancer

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Abstract

Breast cancer is the leading cancer found in females today. Although breast cancer can be broken down into various subtypes, the most prominent type is hormone receptor (HR) breast cancer. Hormones especially effective in females, such as progesterone and estrogen, may stimulate cancer cell proliferation. The four main breast cancer subtypes are HR+/HER2-, HR-/HER2+/−, HR-/HER2+, and HR-/HER2-, each characterized by the presence or absence of certain hormone receptors. HR breast cancers, due to high levels of progesterone and estrogen that promote cell proliferation and human epidermal growth factor receptors, grow by controlling gene transcription, cell division, and migration. Estrogen, a sex hormone primarily found in the female ovaries, binds to its associated receptors throughout the menstrual cycle and pregnancy. Various genes encode estrogen receptors, which regulate the expression of their respective genes. Estrogen causes ER-positive breast cancer growth via the continual binding of the hormone to cancer cell receptors. Conversely, ER-negative breast cancers are tumors that form due to the depletion of estrogen receptors from cancer cells. These cancers are regulated by two receptor types: ERα and ERβ. Recent treatment includes endocrine therapy, surgery, and epigenetic therapy.

Keywords: estrogen, reception, breast cancer, signaling, cancer, endocrine therapy, progesterone signaling

1. Introduction

Breast cancer is characterized by the formation of malignant cells in mammary epithelial tissue, and it is the most common cancer found in females [1]. Over the last three decades, breast cancer has become increasingly common [2]. Through the stimulation of carcinogenic factors, benign tumors and metastatic carcinomas may develop [3]. Carcinogenesis may occur as a result of epigenetic functions, genetic predispositions, and environmental factors such as certain lifestyles, including later pregnancies, a lack of physical activity, and inadequate diets [2, 3].

These factors can be categorized into non-modifiable and modifiable risk factors. Non-modifiable factors include sex, age, race, genetic mutations, and family history,

while modifiable factors include alcoholism, smoking, exposure to dangerous chemicals, and insufficient vitamin supplements [2]. Breast cancer is often found in older women who have late menopause, lack breastfeeding, have a positive family history, or have dense breast tissue [1]. Additionally, genetic mutations that occur on BRCA1 on chromosome 17 and BRCA2 on chromosome 13 are linked to cancer progression [2].

Current treatment for breast cancer includes systemic endocrine treatment, immunotherapies, chemotherapy, and radiation [1]. A lumpectomy, the excision of the tumor from the breast, can be performed through breast-conserving therapy only if the patient can undergo radiotherapy, a treatment that uses radiation to reduce tumor growth and kill cancer cells [4]. Systemic chemotherapy is often recommended for high-risk patients to lower cancer growth [4].

2. Breast cancer diagnosis

Early diagnosis is the best prevention method for breast cancer, but tumors often metastasize and spread to other parts of the body, preventing such prompt diagnosis [3]. Current screenings and imaging allow a pathway to recognize early breast cancer [5]. Mammography, breast ultrasounds, and breast magnetic resonance imaging (MRI) are all procedures for detection [5]. Mammography is the most common screening method, and it allows for efficient, minimal technical setup diagnosis of breast cancer [5]. The simplicity of this method allows mammography to be used in several countries for early diagnosis [5]. Breast ultrasounds provide an enhanced screening of breast tissues, but the quality of screenings depends on the patient's breast size, tissue density, and exposure to previous radiation [5]. Breast MRIs offer the most accurate examination for breast cancer but are the most expensive option [5].

Following these examinations, areas with a lump, microcalcification, or swelling can be identified [5]. The presence of microcalcifications in mammograms is associated with premalignancy and malignancy, but pleomorphic and fine linear microcalcifications are especially associated with these outcomes [6]. Furthermore, they are associated with worse outcomes in eight-year survival rate, lymph node involvement, and tumor width [6].

Due to the multitude of causes and effects of breast cancer, various subtypes exist to categorize the broad area of breast cancer. Such subtypes include metrics such as tumor size, lymph node size, pathologic type, molecular subtype classification, and more recently, receptor status classification [7]. In many cases, microRNA (mRNA) gene expression levels determine molecular subtypes [2]. Receptor status classification considers the role of estrogen receptors, progesterone receptors, and human growth factor receptors (HER2) in breast cancer patients [7].

The recent usage of biosensors and biomarkers as an early detection tool has been extensively studied and is promising on account of their relatively low cost and efficiency [8]. Exosomes and various immune biomarkers have produced promising data for their potential usage in diagnosis and treatment, though full therapies have not yet been tested or developed [9, 10]. MicroRNA expression levels are affected in many cancers, leading to effects on disease progression, severity, and recovery; for these reasons, tracking microRNA levels as a biomarker may prove useful for early detection [11]. Novel apparati, such as that shown by Parchekani et al. [11], can detect microRNAs as biomarkers at a zeptomolar concentration. Furthermore, microwave imaging (MI) techniques have also proven highly promising, as some clinical trials using MI have been successful [8].

3. Hormone receptor breast cancers

Hormone receptor (HR-positive) breast cancer occurs when hormones—especially estrogen and progesterone—attach to their respective receptors and stimulate rapid cancer growth [12]. HR-positive breast cancer requires specific hormones for progression and occurs when cancer cells have embedded proteins that act as signaling molecules that bind to specific hormones [13]. This category of tumors constitutes over 70% of breast cancers [13, 14].

HR-negative breast cancer occurs when cancer cells grow without the presence of these hormones [15]. These cancer cells do not have specific receptors for estrogen, progesterone, or human epidermal growth factor receptor-2 for growth [15]. This cancer involves the absence or overexpression of progesterone, estrogen, and/or human epidermal growth factor receptor-2 in individuals diagnosed with this cancer [7]. Gran et al. have shown that patients with HR-negative tumors have a higher risk of mortality than those with HR-positive tumors [16, 17].

3.1 Mechanism of progesterone in HR breast cancer

Progesterone is an ovarian steroid hormone that plays a crucial role in the female menstrual cycle and pregnancy and is essential for normal breast development [18, 19]. Progesterone receptors (PR) are prevalent among HR-positive breast

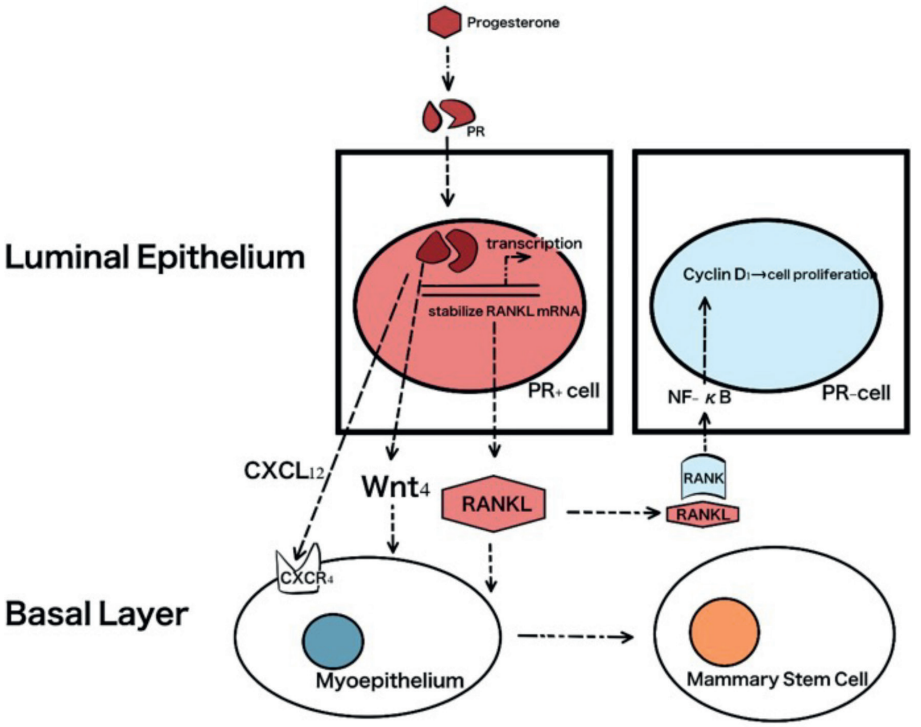

Figure 1.
Progesterone signaling pathway. The reception of progesterone leads to increased nuclear factor κB (NF-κB) ligand (RANKL) through the transcription or stabilization of RANKL mRNA; this increased RANKL leads to the increased proliferation of progesterone receptor-negative luminal cells (adapted from Ref. [19]).

cancers. As progestins, synthetic ligands, and progesterone bind to the PR, a signaling pathway is initiated (**Figure 1**) [18]. This binding initiates PR signaling and regulates gene transcription [20]. As ligands bind to PR in cancer cells, cancer cell proliferation increases. Increased exposure to progesterone increases the risk of breast cancer through the development of HR-negative tumors [19]. Endogenous progesterone, progesterone metabolites, and interactions with estradiol result in a 16% increase in the risk of breast cancer for postmenopausal females [21].

3.2 Role of estrogen in the body

Estrogen is a sex steroid hormone that plays a crucial role in the sexual and reproductive development of females and plays a minor role in male reproductive development [22]. It is essential in various other biological systems, including the vascular, skeletal, and immune systems [22]. Estrogen production in females occurs in the ovaries and may occur in other tissues via androgen synthesis (**Figure 2**) [24]. Estrogen in females can be found in various forms, such as estrone, estradiol,

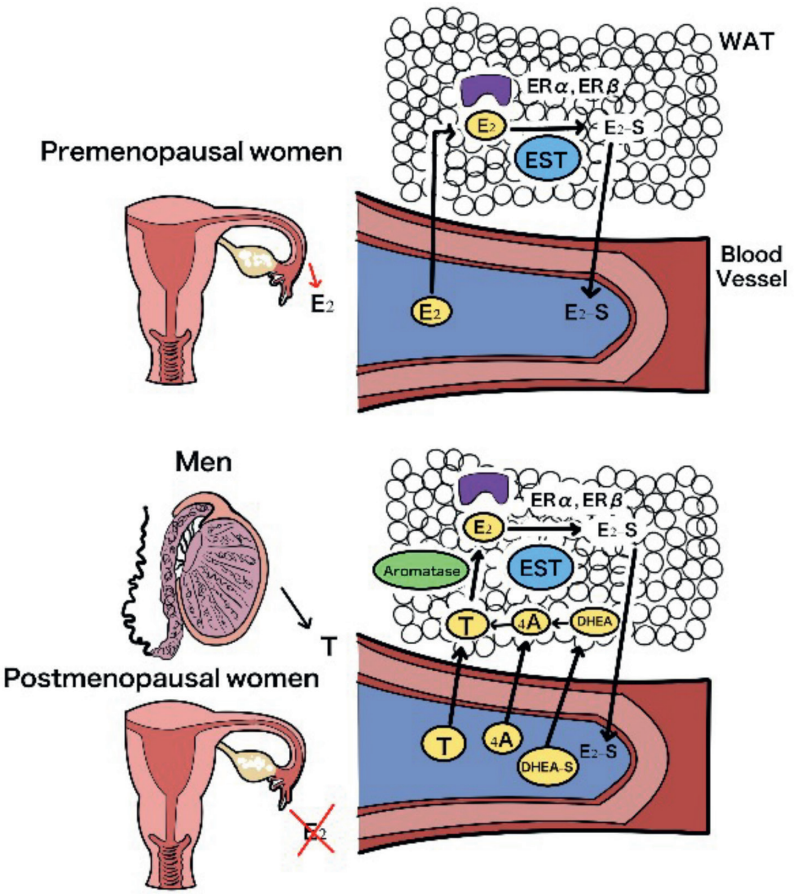

Figure 2.
Origin of circulating and tissue estrogens. In premenopausal women, estrogen is secreted directly from the ovaries. In postmenopausal women and in men, it is instead produced from androgenic precursors such as testosterone (adapted from Ref. [23]).

and estriol, with each molecule varying in structure and activity level throughout the female reproductive cycle [25]. These estrogens act as signaling molecules that bind to receptor molecules during different periods of the menstrual cycle and pregnancy [25].

In postmenopausal females, estradiol is not produced as abundantly due to the ovaries failing [23]. Instead, estradiol is produced in extragonadal sites, including the breasts, brain, muscles, bones, and adipose tissues [23]. In men, circulating estrogens are not produced; instead, biosynthesis occurs via circulating androgens [23]. This leads to the regulation of estrogen through tissue metabolism or inactivation of circulating estrogen, specifically through tissue estrogen sulfotransferase (EST). This enzyme prevents the binding of estrogen to their respective receptors [23].

4. Mechanism of HER2 in HR breast cancer

HR-positive breast cancer may also arise due to the upregulation of the human epidermal growth factor receptor (HER2), a membrane tyrosine kinase receptor [26, 27]. HER2 overexpression promotes cancer cell growth by promoting cell division, differentiation, and migration [28]. Phosphorylated tyrosine kinases activate HER2, leading to eventual cell cycle progression and proliferation [28]. The HER2 is part of a signaling pathway composed of three layers: membrane receptors and their ligands, protein kinases, and transcription factors (**Figure 3**) [29]. The first layer of receptors takes in the

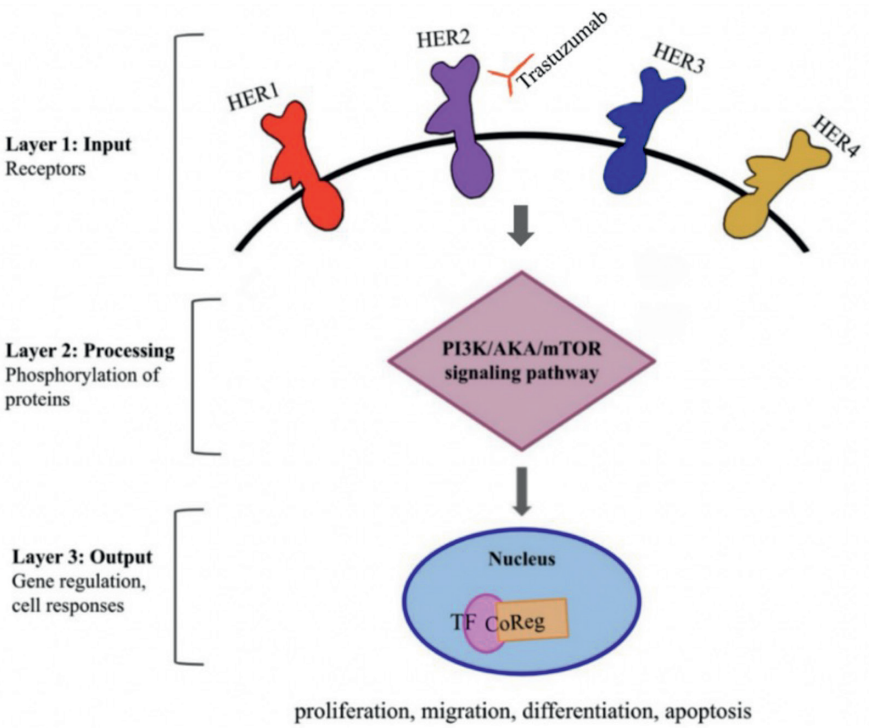

Figure 3.
HER2 signaling pathway. The pathway consists of three layers in order to regulate proliferation, migration, differentiation, apoptosis, and angiogenesis (adapted from Ref. [29]).

signal, the second layer involves the phosphorylation of protein kinases that transmit signals to the nucleus, and the final layer regulates genes for proper cellular function [29]. Although the input layer consists of four tyrosine kinases HER1–4, breast cancer research primarily focuses on the HER2 tyrosine kinase receptor [29].

HER2 overexpression occurs through HER2 amplification, which increases cell proliferation rates, lowers estrogen and progesterone levels, promotes aneuploidy, and causes resistance to endocrine therapy [28, 29]. Due to this resistance, HER2-targeted therapy controls the expression of growth factors [30]. Trastuzumab is a cancer drug developed to target HER2 protein kinases in the early stages of breast cancer by attaching to HER2 proteins to prevent cancer cell growth and proliferation (**Figure 3**) [29, 30].

Although HER2 and PRs in cancer cells can cause hormone receptor breast cancers, estrogen receptor signaling in cancer cells is the most prevalent cause of breast cancer. Impaired estrogen receptors and signaling are responsible for a high percentage of breast cancer cases [7].

5. Estrogen receptors and signaling

Estrogen receptors (ERs) are nuclear proteins found in cells that regulate the expression of specific genes [31]. Various genes encode these receptors, but they can be expressed in the same tissues [31]. Dimer formation is important for the function of estrogen receptors to regulate gene expression, as it is for many steroid hormones [32]. Circulating estrogen is regulated by two estrogen receptors: ERα and ERβ (**Figure 4**) [31]. These receptors can bind to agonist, agonist–antagonist, or full antagonist molecules [31].

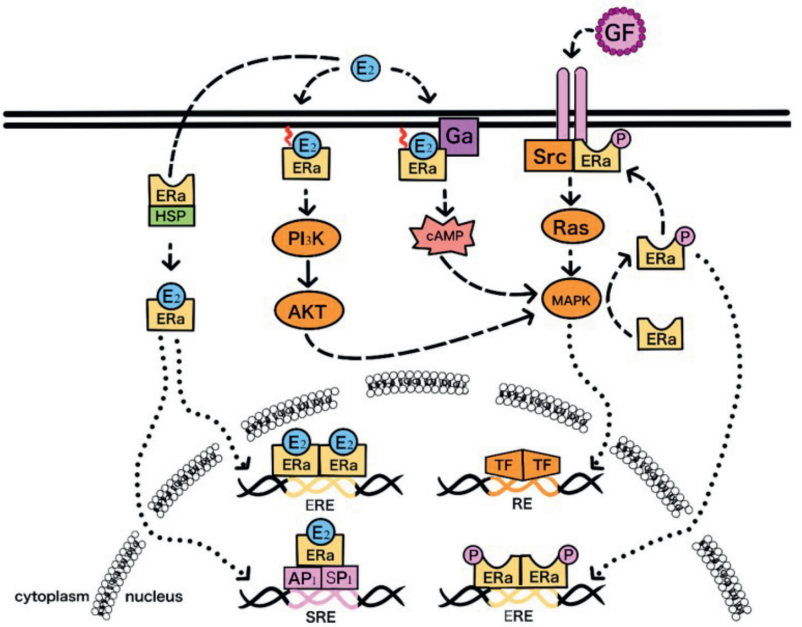

Figure 4.
ERα signaling pathway. The regulation of transcription of target genes is completed in the genomic activity of ERα, while the activation of transcription factors relating to cell proliferation and survival is done in the non-genomic activity of ERα (adapted from Ref. [33]).

Estrogen regularly binds to its receptors in breast cells; however, when cancer cells are present with the same receptors, estrogen binds to these proteins and causes rapid cancer growth in the affected individual [23]. ERs function as ligand-activated transcription factors; when the ligand attaches to the receptor, a conformational change occurs in the protein [34]. The resulting transcription factor is translocated to the nucleus, where it attaches to estrogen response elements—which are specific DNA sequences—to regulate the transcription of target genes [34]. Further, mutations may occur in the receptors that cause them to lose the ability for ligands to bind and thereby the ability for transcription to occur.

Estrogen receptors contain different distinct amino acid compositions that allow for specific 17β-estradiol [E2] signaling [35]. Estrogen receptor-ligand-binding domains (LBDs) are made up of a three-layer α-helical fold [35]. Estrogen receptors contain unstructured carboxyl-terminal extensions—an extra domain—to maintain their structure [35]. ERα LBDs have a helical arrangement of 12 helices that create a site for ligands to bind [35, 36]. The LBD domains in ERα allow for the regulation of transcription, coactivators, dimerization, and stability [35]. ERα also contains a globular LBD that holds hormone-binding and coregulator interaction sites [36]. E2, as a natural ligand, binds easily in a hydrophobic environment [36]. Such hydrophobic interactions between molecules and estrogen receptor helices allow for the formation of specific LBD structures, subsequently allowing estrogen to bind (**Figure 5**) [36, 37]. Synthetic ligands are currently being developed to overcome various diseases [37]. ERβ has a similar structure to an arbitrary coil, but little is known of the function [35].

Estrogen receptor-DNA binding domains (DBDs) are usually found central to estrogen receptors and provide the ability to bind to estrogen response elements (EREs) [35]. EREs are palindromic DNA sequences that allow estrogen receptors to bind as a transcriptional regulator [2]. Zinc finger domains regulate ERE interactions and dimerization in DBDs [36]. Three amino acids found in the zinc finger domains

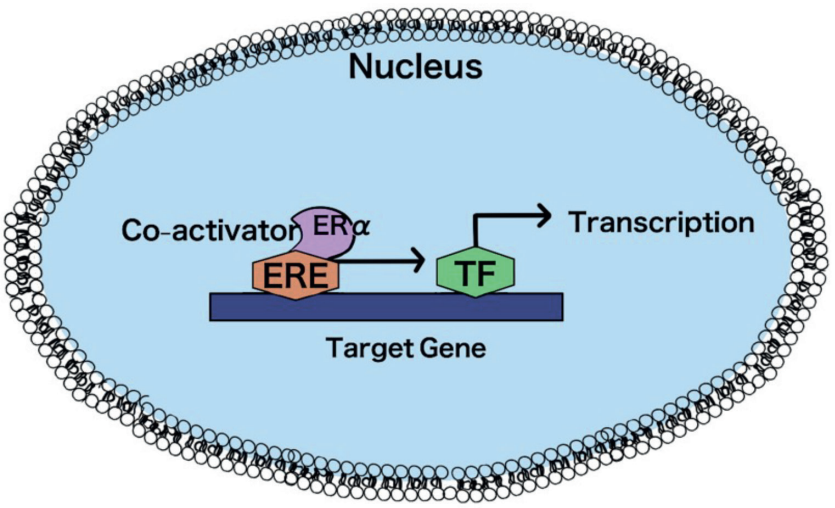

Figure 5.
17β-estradiol [E2] signaling. In this pathway, an ER bound to E2 interacts with a G-protein (GP) that results in kinase activation, further leading to co-regulatory proteins (CR) and transcription factors (TF) alongside the ER complex altering gene expression (adapted from Ref. [36]).

determine the interaction with EREs and the glucocorticoid response element (GRE) [36]. Depending on the amino acids, the transcriptional activity can be mediated by EREs or GREs [36].

Following reception and dimerization, the estrogen receptor complex translocates to the nucleus; though the specific mechanism through which this translocation occurs is currently unclear, the general transport of nuclear hormone receptors to the nucleus is facilitated by the interactions between nuclear localization signals with importins and microtubule-associated motor proteins [36].

Transcriptional activity of estrogen receptors occurs through the interactions between two activation function domains: AF1 and AFR2 [36]. AF1, located in the N-terminal domain, can function without the presence of a hormone, and AF2, located in the LBD, only functions in the presence of a hormone [36, 38]. In a study conducted using mice by Couse et al., ERαAF2 activity was observed, and the results suggested the essential role of AF2 in E2 transcriptional activity [38, 39].

G protein-coupled estrogen receptors (GPERs) are crucial to breast cancer progression, in which they bind to estrogens and other molecules; this binding results in the modulation of genes involved in cell proliferation, metastasis, apoptosis, and migration [40]. Specifically, G protein-coupled estrogen receptor 1 (GPER-1) in patients with triple-negative breast cancer is associated with shorter overall survival and relapse-free survival in premenopausal patients [40]. GPER-1 and ERβ facilitate estrogen responsivity in triple-negative breast cancer despite the lack of ERα expression [41]. Furthermore, measuring GPER expression or its cellular distribution may provide new avenues in diagnosis for ER or estrogen-related diseases [42].

6. Estrogen receptors in breast cancer

6.1 ER-positive and ER-negative breast cancers

ER-positive breast cancer is the most common form of breast cancer (**Table 1**) [33]. Estrogen acts as a signal for tumor growth [33]. ER breast cancers, also known as luminal breast cancers, are dependent on the presence or absence of estrogen [43]. Breast cancer can be classified into subtypes based on immunohistochemical staining regarding estrogen, progesterone, and HER2 levels (**Table 1**) [43]. Using microarray-based expression profiling, tumor subtypes can be analyzed to establish molecular classification [43, 44]. ER-positive breast cancers can be further divided into subtypes luminal A and B [43, 44]. The luminal A subtype is PR positive and HER2 negative (HR+/HER2-) [44, 45]. Due to lower proliferation rates, the luminal A subtype has a

	ER-positive breast cancer		**ER-negative breast cancer**	
	Luminal A	**Luminal B**	**ER-negative**	**Triple negative**
HER2	Negative	Negative	Positive	Negative
Hormone receptor	Positive	Positive/Negative	Negative	Negative
Proportion of breast cancer cases (%)	60	10	20	10

Table 1.
Breast cancer subtypes. Proportion of breast cancer cases organized into subtypes: Luminal A, Luminal B, HER2 positive, and triple negative (adapted from Ref [33]).

lower relapse rate after treatment; this lower relapse rate is indicated by low levels of Ki67, the proliferating cell nuclear antigen [44]. The luminal A subtype has high levels of ER and high expression of luminal epithelial cytokeratins 8 and 18, ER1 markers, and the LIV1, FOXA1, XBP1, GATA3, and BCL2 genes [44]. Furthermore, luminal-A breast cancer has a relatively low expression of genes associated with cell proliferation compared to its luminal-B counterpart, leading to such cancers being slow-growing and low-grade and to such cancers having the best prognosis [2]. Luminal B cancers are PR-negative and HER2-positive or negative (HR-/HER2+/−) [43–45]. The luminal B subtype has higher proliferation rates, indicating high levels of Ki67 [43]. This subtype has increased expression of the proliferation-related genes GGH, v-MYB, LAPTMB4, NSEP1, and CCNE1 [44].

There is evidence that androgen receptors (AR) play a role in the formation and proliferation of cancer cells [33]. In ER-positive breast cancer, AR can both stimulate and hinder the growth of cancer cells. AR has also been found to use Src protein kinase and PI3K—a pathway discussed later in this review—to promote the proliferation of ER-negative cancer cells [33].

ER-negative breast cancers are tumors that form due to the depletion of estrogen receptors from cancer cells [33]. Breast cancer grows rapidly in the absence of estrogen and thus there are fewer cancer prevention treatments for this disease, leading to patients diagnosed with ER-negative breast cancer generally having a lower chance of survival due to both this rapid growth and poor tumor differentiation [46]. Due to this, ER-negative breast cancer tumors are commonly identified at a young age [47]. Studies from Sharma et al. have shown that epigenetics—how environment and behavior affect and change a heritable phenotype but not DNA itself—may affect the onset of ER-negative breast cancer [46, 47]. Epigenetic factors include poor diet quality, which can stimulate stress through the low intake of fruits and vegetables as well as low intake of vitamins, decreasing DNA methylation [47].

ER-negative cases are commonly paired with PR expression [48]. For this reason, estrogen resistance can be classified into two categories: de novo and acquired [48]. De novo resistance occurs in the absence of both estrogen receptors and progesterone receptors in cells [48]. In these cases, the lack of estrogen in the body may be caused by long-term activation of growth factor signaling pathways that are no longer able to produce any signal at all or one that is sufficient to produce successive molecules [48]. On the other hand, acquired resistance occurs when there is initial estrogen responsiveness but later unresponsiveness [48]. Several drugs that attempt to battle the relapse of ER-positive breast cancer can unexpectedly cause ER-positive tumors to develop resistance to that drug and become ER-negative tumors [48].

HER2-positive cases occur when tumors are ER negative and PR negative but HER2 positive (HR-/HER2+) (**Table 1**) [33]. The HER2 receptors are overactive in these cases, causing cell proliferation through several signaling pathways such as PI3K/AKT, as described later in this review [33]. HER2 ER-negative breast cancers are more aggressive due to increased development of metastasis [33].

Triple-negative breast cancer (TNBC) is ER, PR, and HER2 negative (HR-/HER2-) [43–45, 49, 50]. Triple-negative breast cancer is usually basal subtype tumors that have an unfavorable prognosis [43]. These tumors are treated with chemotherapeutic agents due to difficulties with using a targeted drug for endocrine treatment [43]. Basal-like subtypes have higher rates of metastasis to the brain and lungs due to the growth of infiltrating ductal tumors [44]. Although most are established as basal-like through molecular classification, not all TNBCs are basal-like [43, 44].

Data shows that TNBC patients have larger tumor sizes, greater proliferation rates, and more aggressive metastasis than other breast cancers [50].

6.2 Estrogen receptor type: ERα

ERα is an estrogen receptor type found in the mammary epithelium [51]. ERα consists of an N-terminal domain, a DNA-binding domain, and a C-terminal ligand-binding region where the estrogen binds [51]. The C-terminal ligand-binding region consists of an antiparallel α-helical fold that maintains a ligand-binding cavity for the receptor [31]. The gene for this receptor is on q24-a27 of chromosome 6 [31]. ERα travels to the nucleus once estrogen is bound and regulates gene transcription of its associated genes [34]. Transcription may be disrupted by mutations in dimerization [31]. Specifically, ERα specializes in regulating the genes that control cell proliferation, differentiation, and migration [52]. ERα is modulated by extranuclear signaling, coregulators, post-translational modifications, and microRNA and can be affected by deregulated expressions of these factors [52]. This fragility makes the mammary epithelium easily susceptible to cancer cell development [52].

One example of a coactivator of ERα is CDKN1A-interacting zinc finger protein 1 (CIZ1), which responds to circulating estrogen [53]. CIZ1 is considered a coactivator rather than an oncoprotein because no mutations of CIZ1 have been found to cause cancer [54]. CIZ1 increases the expression of estrogen in target genes by binding to estrogen receptors in cancer cells [54]. The coactivator increases the activity of cyclin-dependent kinase 2 (CDK2) by binding to estrogen-induced protein DLC1 [54]. CDK2, in turn, causes the rapid growth of breast cancer cells [54]. Several coactivators and ERα activate transcription factors in the presence of estrogen on a target gene (Figure 6) [53].

Extracellular signals are transmitted through G-proteins that are linked to membrane ERα [55]. The deregulation of specific signaling pathways begets disorder in cell interactions and development, contributing to breast cancer in individuals [52]. In a study conducted by Manavathi et al. [53] to determine the role of ERα, deletion of this receptor causes the ductal system to fail to expand in mice due to the lack of regulation of circulating estrogen in their systems. After the maturation of the mice, however, there was little to no mammary gland development in female mice [53]. ERα interaction with target genes may occur through estrogen response elements or DNA transcription factors [53]. A receptor may either positively or negatively mediate target gene transcription [53].

Extranuclear signaling occurs through ERα activation by circulating estrogen, which causes rapid cytoskeletal reorganization and the development of filopodia and ruffles in motile cells [56]. ERα extranuclear signaling affects breast cancer motility as it promotes the stimulation of Src kinase, mitogen-activated protein kinase, phosphatidylinositol 3-kinase (PI3K), and protein kinase C [56]. Breast cancer cell migrations occur through kinases activated by ERα extranuclear signaling while other signaling pathways play roles in the invasion and metastasis of these cells [56]. ERα works with growth factor receptors such as HER2 to reorganize the cytoskeleton in breast cells; the dysregulation of the growth factor in cells can promote cytoplasmic sequestration of ERα [56]. ERα may be methylated through post-translational modifications by methyltransferase G9a [56]. Depletion of G9a in breast cancer cell lines decreases estrogen signaling and slows down cell proliferation and transformation in cells [56]. Increases in extranuclear signaling may be responsible for breast cancer cell migration and metastasis [56].

In ER-positive breast cancer, estrogen initiates the PI3K/AKT/mTOR signaling pathway [55]. When it is targeted, the PI3K/AKT- protein kinase Cα signaling pathway activates, which results in the increased expression of the signaling pathway as well as tumor invasion and metastasis, causing cancer to spread [55, 57]. Additionally, breast cell apoptosis occurs while cancer cells proliferate [55]. Cyclic adenosine monophosphate (cAMP) levels increase as a result of the activation of these signaling pathways, causing a rapid increase in the presence of estrogen [55]. Consequently, the C-terminal of ERα is activated and interacts with signaling molecules to amplify its signaling pathway [55]. This activation increases HER2 levels and epidermal growth factor receptor levels, causing further cancer growth [55]. Similar disease progression by the overexpression of ERα has been linked to other cancers such as prostate cancer [55].

Rajhans et al. have shown the retention of ERα in metastatic tumors, indicating the correlation between ERα-positive tumors and metastasis [56, 58]. Metastatic ER-positive cancers commonly spread to the bone, bone marrow, lymph nodes, lungs, liver, and skin [33]. Specifically, studies by Koenders et al. have shown a correlation between ERα-positive tumors and bone metastasis in breast cancer patients [56, 59]. Metastasis occurs when tumor cells migrate from the stroma into the bloodstream and progress through epithelial-mesenchymal transitions (EMT) to increase the mobility of cancer cells [60]. Once in the bloodstream, tumor cells attach to organs, spreading cancer to another part of the body [60].

Studies by Borough et al. show the correlation between rapidly spreading tumors and oncogenic signaling pathways that contribute to anabolism and metabolism [17, 60]. Lowered levels of glutaminolysis, glycine, lactate, and glutamate in ER-positive tumors indicate the role of estrogen receptors in tumor metabolism [60]. The PI3K/AKT/mTOR signaling pathway works in conjunction with estrogen receptors to allow for tumor stabilization [60]. The process of glycolysis may be altered in periods of high glucose conditions by estradiol, and glucose availability may also alter the programming of metabolism by ERα [60].

6.3 Estrogen receptor type: ERβ

ERβ, like ERα, is an estrogen receptor type found in the nuclear transcription factor family [49]. It is encoded by the ESR2 gene, which has eight encoding exons and is found predominantly in breast epithelial cells [49]. As with ERα, ERβ has an N-terminal activation function-A that stabilizes ligand-independent transcription as well as multiple domains for ligand binding [49]. ERβ, unlike ERα, does not target the uterus or mammary gland but instead regulates signaling in the ovary, immune system, and prostate [61]. The N-terminal domain contains a repressor function in ERβ that decreases ERβ-mediated activity; this domain plays a role in regulating interactions between ERβ and cellular pathways in the body [61]. In the presence of estradiol, ERβ inhibits transcription, while ERα activates transcription on AP-1-driven promoters [61].

As the levels of ERβ increase, levels of cell proliferation decrease, serving as a tumor suppressor [62]. Breast cancers display decreased levels of ERβ [62]. The downregulation of these estrogen receptors exhibits EMT in cancer cells and prevents cancer cell migration [62]. This downregulation is done by inhibiting receptor tyrosine kinases and activators of transcription signaling pathways of cancer cells [63]. ERβ carries tumor-suppressor characteristics by inhibiting transforming growth factor (TGF) signaling and decreasing the expression of genes in the extracellular matrix, cell invasion, and vitamin metabolism [63]. ERβ has also been shown to play

an inhibitory effect on cell cycle progression [63]. By decreasing levels of ERβ, cancer cells elevate cyclin levels needed for cell cycle progression and cause rapid proliferation and growth [63].

Studies by Paruthiyil et al. have identified a network of genes in the G2 stage of breast cancer cells [63]. Targeting this specific stage in the cell cycle, ERβ represses cyclin D1, cyclin A, and c-myc gene transcription while increasing the expression of Cip1 and Kip1 to inhibit cell proliferation before progressing toward mitosis [63, 64]. Cyclin-dependent kinases, regulatory enzymes necessary for the progression through the cell cycle, are also inhibited by ERβ [63].

ERβ isoforms in mRNA can form through alternative splicing of exons 8 and 9, deletion of a coding exon, or the use of untranslated exons [65]. ERβ isoforms are differentially expressed and associated with breast cancer tumor growth [66]. In an experiment conducted to observe the role of ERβ in therapy for triple-negative breast cancer, ERβ isoforms ERβ2/ERβ5 and ERβ1 were shown to serve different functions in cancer cells [67]. The different outcomes of the subgroups are due to the various mRNA and protein expressions from different ERβ isoforms [66].

The role of ERβ is versatile in providing an antiproliferative role when in the presence of ERα. At the same time, some data correlate the growth of breast cancer with ERβ as in experimental studies by Förster et al. [68, 69]. Through treatment of mice in a 48-hour study, they presented the mediation of tissue-specific cell proliferation with colocalization of ERβ in mammary epithelial cells (Couse et al.) [38, 39]. However, this data presented that ERβ1, the functional isoform, did not inhibit the proliferation of cells in some circumstances [38]. The presence of ERβ in some breast cancer tumors indicates the lack of a role of ERβ in halting cancer growth [65]. Rody et al. have associated the methylation of ERβ promoters in cancer cells with the depletion of ERβ in breast cancer cells [65, 70]. In *in vitro* studies, the lack of ERβ in breast cancer tumors supports that ERβ is a tumor suppressor gene [65]. The role of ERβ is continually being studied due to the duality it presents in various studies [68]. Due to this uncertainty, ERα is measured to determine the treatment and identification of the type of breast cancer for a patient [65].

6.4 Difference in receptor types

During the progression of tumors, ERβ levels decrease, serving as a tumor suppressor [61]. This suggests a role opposite of ERα which promotes cancer cell proliferation [61]. Malignant tumors show a higher expression of ERα and a lower expression of ERβ; however, as discussed in a previous study, the presence of ERβ levels in breast cancer patients is still being studied [38, 65, 71]. Due to this, ERβ expresses an anticancer effect and is used in therapies for breast cancer treatment [63]. ERα and ERβ levels are responsible for the signaling pathways that mediate cell proliferation and therapy response [72, 73].

The interaction between these receptor types can affect the transcription and downregulation of breast cancer cells [73]. Transcription is upregulated in the presence of the AP-1 binding element with ERα but downregulated in the presence of estradiol with ERβ [74]. Although similar in their structure and their role in controlling estrogen signaling by binding to an ERE, the function of each receptor differs [71]. The ligand-binding domain of both receptors also differs, allowing for subtype selectivity [71]. Other factors such as transcriptional activation and cofactor interactions cause different effects of ERα and ERβ in tissues [71].

7. Recent progress: Treatment

7.1 Endocrine therapy

Endocrine therapy (ET) is a common form of treatment used for estrogen receptor-positive cancers in conjunction with surgery [31]. This therapy can be categorized into three groups: ovarian function suppression, estrogen receptor modulators and down-regulators, and aromatase inhibitors [31].

Ovarian function suppression (OFS) is a form of therapy used to decrease and stop the production of estrogen by the ovaries [31]. This therapy lowers the level of estrogen in the body and prevents the overexpression of estrogen that causes cancer cells to grow [75]. Through luteinizing hormone-releasing (LHRH) hormone agonists or radiation therapy, OFS can be used to treat tumors [75]. LHRH targets G-protein coupled receptors in pituitary glands to reduce ovarian hormone production in the long term for premenopausal females [75]. OFS is used with adjuvant endocrine therapy to treat ER-positive breast cancer patients [76].

Selective ER modulators (SERMS) and down-regulators (SERDS) are also used to stop estrogen receptor function and signaling [31]. A common selective ER modulator, Tamoxifen (TAM), is used to treat both pre- and postmenopausal patients [31]. TAM acts as a competitive receptor inhibitor—blocking estrogen from binding in the site—in breast tissues by blocking the signaling of estrogen receptors and is commonly used for premenopausal women [49, 76]. SERMS are dependent on the activity of an estrogen receptor agonist or antagonist to determine the complexity of the compound [77]. SERMs use target-site specificity to interact with either of the two subunits that an estrogen receptor is composed of [77]. SERDs act as ER antagonists, blocking the function and signaling of receptors by degradation [31]. Those who are resistant to first hormone therapy—commonly those with luminal metastatic breast cancer—have been treated using the SERD fulvestrant [78]. Although oral administration is currently being developed, fulvestrant is usually administered through an intramuscular injection in postmenopausal women [78]. Approved by the FDA in January 2023, elacestrant is an oral SERD for ER-positive breast cancers [33, 79]. The EMERALD trial conducted by Aditya Bardia et al. found an improvement in phase III patients in using elacestrant for hormone therapy with an overall 30% reduction in the progression of the cancer in the study population [79].

Aromatase inhibitors block aromatase enzyme activity by decreasing the circulation of estrogen in the bloodstream, thereby reducing the frequency of estrogen receptor binding [31]. However, aromatase inhibitors have been shown to only be effective in postmenopausal females [31]. These inhibitors do not function in females whose ovaries are active, as they increase estrogen production [31]. The STAGE study conducted by Masuda et al. has shown that 70.4% of postmenopausal patients who undergo aromatase inhibitor and OFS therapy have a response to treatment [31, 80].

Endocrine therapy has proven to be beneficial for ER-positive patients; however, mutations present in ERα can lead to endocrine resistance [55]. ERα is affected by post-translational modifications, often getting degraded through ubiquitination [55]. Endocrine resistance can cause ER-positive patient relapse due to changes in protein expression from ERα alterations [55]. One mutation that exists is the Y537S mutation that causes endocrine resistance against Tamoxifen in MCF7 cells, according to

studies performed by Fiorillo et al. [81]. Results show that the Y537S mutation creates Tamoxifen resistance through refined mitochondrial metabolism and increased levels of glycolysis due to increased mitochondrial oxygen intake [81].

7.1.1 Endocrine therapy induced osteoporosis

Aromatase inhibitors are chosen as the primary treatment for postmenopausal women by blocking the production of estrogen from androgens [80, 82]. Endocrine therapy is used to inhibit the growth of tumor cells by reducing the levels of estrogen. This therapy will prevent estrogen from binding to estrogen receptors on tumor cells [82]. However, the reduced estrogen levels stimulate osteoporosis—the loss of bone density with decreased estrogen levels—in older women and hinder bone formation and growth [82]. This development is commonly induced by the anti-estrogenic drugs and aromatase inhibitors mentioned previously [82].

There has been recent progress in the use of cyclin-dependent kinase inhibitors for these women. Specifically, CDK4 and CDK6 inhibitors prevent tumor cell growth and proliferation [82]. Cyclin-dependent kinases are key in controlling the progression of the cell cycle. Normally, the cyclin D-CDK4/6 complex phosphorylates Rb, the retinoblastoma protein, allowing for the release of E2F, which binds to promote growth. Without the presence of CDK4/6, these cells cannot progress from the G1 to S phase, preventing the growth of these cells [83]. CDK4/6 inhibitors include Abemaciclib, Palbociclib, Ribociclib, and Trilaciclib [82]. These inhibitors are mostly used for HR+/HER2- patients, and specifically palbociclib is used in conjunction with aromatase inhibitors to treat postmenopausal patients [83]. However, as with many other breast cancer treatments, acquired resistance to CDK inhibitors can still occur, necessitating future research into CDK inhibitors and how such resistance arises [83]. One novel CDK inhibitor, Dalpiciclib, has shown promise for lengthening progression-free survival of HR+/HER2- patients that have been heavily pretreated; furthermore, it also displays some synergistic effects when used in combination with other therapies [84]. Nevertheless, further research into CDK inhibitors as a therapeutic option may prove beneficial [83].

7.2 Surgery

Surgery is used to treat ER-negative cancers to directly target tumor growth [85]. Surgery for cancer prevention allows for enhanced tissue retrieval and local and regional control of solid cancers [85].

HR-negative breast cancers are commonly treated through lumpectomy [86]. Lumpectomy treats centrally located tumors on the breast through surgical removal of the tumor and surrounding tissue [86, 87]. This surgery is performed to conserve the breast and remove the cancerous tumor and tissues. Radiation therapy is used after lumpectomy to reduce the recurrence rate of breast cancer [88]. Although these surgeries are low risk, in the case that a lumpectomy fails, a re-excision or a mastectomy may be required [87]. A mastectomy removes all breast tissue due to the large size of tumors and the spread of the cancer. In low-income countries, mastectomy is the preferred surgical treatment due to the late stage of breast cancer diagnosis and the prevalence of the disease [85].

7.3 Epigenetic therapy

Epigenetics is known to play a substantial role in many normal developmental functions, but its role in cancer initiation and development has only recently been

researched [89]. In breast cancer, epigenetic modifications such as rapid DNA methylation, cytosine-phosphate-guanine (CpG) promoter methylation, and histone modification may lead to its initiation, promotion, or metastasis; furthermore, other epigenetic functions may result in the silencing of tumor suppressor genes, contributing to epigenetics' role in cancer development [89, 90].

Epigenetic drugs (epi-drugs) are chemical agents that inhibit these epigenetic changes [89, 90]. The most common method is for epi-drugs to reactivate tumor suppressors and DNA repair elements by inhibiting DNMTs-DNA methyltransferase which inhibits CpG island methylation [90, 91]. The drug, DNMTi creates an apoptotic response for cancer cells [90, 91]. Similarly, histone deactetylases (HDACs) are inhibited by the HDACi epi-drug, which normalizes histone acetylation through decreasing activation of zinc-dependent enzymes [91].

Consequently, many epi-drugs have been developed to tackle breast cancer from the epigenetic angle; however, many so far have been confined only to hematological malignancies and have had little success with solid tumors [92]. Preclinical and clinical evidence supports that epi-drugs may have a synergistic effect when used in conjunction with radiotherapy, immunotherapy, and endocrine therapy [92]. The most common types of epigenetic therapy for breast cancers are histone deacetylase inhibitors, DNA methyltransferase inhibitors, and RNA inhibitors, which ultimately aim to restore normal expression of tumor suppressor genes [93]. Some studies have shown that such therapy may be especially effective for hormone-resistant breast cancer [93].

DNMTi and HDACi epi-drugs have low specificity, causing scientists and providers to look for a more selective option [94, 95]. A lysine-specific demthylase 1 (LSD1) inhibitor called Iadademstat has made progress in proving to be an epigenetic treatment for breast cancer by targeting SOX-2 [94, 95]. SOX-2 is prominent in differentiating adult cells and therefore, in high levels, proliferates cancer cells [95].

8. Conclusion

Estrogen is a hormone that can control breast cancer growth through the presence or depletion of it in the body. Regularly, estrogen circulates in the bloodstream as produced by the ovaries and aids in the progression through the menstrual cycle and pregnancy. The role of estrogen receptors and signaling in breast cancer is crucial to understanding hormone receptor breast cancer, as ER breast cancer comprises two-thirds of all breast cancer cases. ER-positive breast cancers are attributed to the necessary presence of estrogen in the body for cancer cell growth to progress. Estrogen receptors and their signaling can cause cancer cell proliferation and tumor growth.

ER-negative breast cancers occur when in the absence of the hormone, cancer growth occurs, as these cells cannot proliferate in the presence of estrogen. Metastasis of breast cancer may also occur as a result of both cancers spreading to several places in the body. ERα and ERβ are estrogen receptor types that bind with estrogen to function in the cell. Although these receptor types are similar in structure, the function differs in that ERα mediates estrogens in the mammary gland, promoting growth, while ERβ inhibits cell proliferation.

One future treatment includes tumor cell apoptosis, which would slow tumor progression, potentially making it an effective tool to treat ER-breast cancer [96]. Lok et al. have developed a potential method of BCL2 inhibition, which is an apoptotic mechanism, to slow the progression of ER-positive breast cancer [96, 97].

By inhibiting the activity of BCL2, the apoptotic processes in tumor cells can be restored [96]. Similar drugs are being developed to target ER-positive tumor cells [96].

The reception and signaling of estrogen receptors play a key role in the development of breast cancer. Treatments using endocrine therapy and other therapies are being developed to medicate those affected by ER breast cancer.

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Conflict of interest

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