The biological role of estrogen receptors α and β in cancer

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Abstract

The temporal and tissue-specific actions of estrogen are mediated by estrogen receptors α and β. The ERs are steroid hormone receptors that modulate the transcription of target genes when bound to ligand. The activity of these transcription factors is regulated by a variety of factors, including ligand binding, phosphorylation, coregulators, and the effector pathway (ERE, AP1, SRF). The end result of target gene

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transcription is to modulate physiological processes, such as reproductive organ development and function, bone density, and unfortunately contribute to the growth and development of breast and endometrial cancer. The complex biological effects mediated by ERα and ERβ involve communication between many proteins and signaling pathways. An ultimate goal of current research is to enhance the value of the separate estrogen receptors as targets for therapeutic intervention.

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1. Introduction

Estradiol (E2) regulates the growth, differentiation, and physiology of the reproductive process through the estrogen receptor (ER). E2 also affects other tissues, such as bone, liver, brain and the cardiovascular system. Because of the functional diversity displayed by estrogens through the ER, much of the current interest in understanding the basis of ER actions at the molecular level is focused towards the goal of therapeutic intervention [1,2].

One of the earliest studies reporting a relationship between breast cancer and ovarian hormones described breast tumor regression after removal of the ovaries [3], the major site of estrogen production in premenopausal women. However, only one in three women respond to oophorectomy [4]. The explanation for these observations became clear when the ER was discovered [5]. In the late 1960s and early 1970s, the ER was initially used as a predictor of breast cancer response to endocrine ablation. Tumors that were ER rich were more likely to respond to endocrine therapy than if the tumor was ER poor [6,7]. In the mid 1970s, before adjuvant therapy became the standard of care, the ER was viewed as a prognostic indicator after surgery, with ER-positive patients responding better than ER negative patients [8]. From the 1970s to the present day, the ER has evolved to be the most effective target for breast cancer therapy. Interactions between E2 and the ER can be blocked using a variety of agents. Selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene, are competitive inhibitors of E2 at the ER and display agonist or antagonist behavior depending on the tissue [9]. Pure antiestrogens, exemplified by fulvestrant (ICI 182,780), only produce antagonist effects and are proving to be useful in treating advanced breast cancer [10,11]. Aromatase inhibitors, such as anastrozole, that block the conversion of androstenedione to testosterone to estrone and estradiol, respectively, are a particularly interesting new approach to breast cancer treatment as the compounds appear to increase efficacy and reduce side effects compared with tamoxifen [12–15]. The optimal combinations and sequential orders of treatment continue to be investigated in clinical trials.

Although the primary focus of research for the first 30 years (1960–1990) has been on the role of steroid receptors in reproductive functions and breast cancer, there is reason to believe that there are opportunities to design new molecules targeted to novel sites dominated by one ER or the other. This is especially true since the publication of the Women’s Health Initiative did not demonstrate an overall health benefit for women taking hormone replacement therapy (HRT) [16]. Positive aspects of HRT include a decrease in the rate of bone density loss, a decrease in total and LDL cholesterol, and a protective effect against colon cancer. However, the risk of breast cancer is increased in HRT users [17]. The challenge now is to dissect the individual roles of ERα and ERβ as transcription factors that participate in normal and aberrant physiological processes. Clearly, the goal will be a menu of multifunctional medicines that can be used singly or in combination to treat and prevent a range of diseases associated with menopause or reproductive function.

2. Isoforms, domains, ligand binding characteristics and expression of ERα and ERβ

The therapeutic targets estrogen receptors α (ERα) and β (ERβ) are members of the nuclear receptor superfamily of transcription factors. Other members of this family include thyroid receptor, Vitamin D receptor, retinoic acid receptor, and other steroid receptors such as the glucocorticoid receptor, androgen receptor, progesterone receptor and mineralocorticoid receptor.

ERα was the first estrogen receptor cloned and it was isolated from MCF-7 human breast cancer cells in the late 1980s [18–20]. In accordance with its role as a transcription factor, this 66 kDa ERα localizes primarily to the nucleus. A 46 kDa isoform (hERα46) that lacks the first 173 amino acids of the 66 kDa form of ERα has also been preliminarily characterized [21]. In addition, several ERα splicing variants have been described [22,23], but whether they are expressed as proteins that have a biological function remains unknown.

Ten years later, ERβ was cloned from rat prostate using degenerate PCR primers [24]. Mouse [25] and human [26–28] forms of ERβ have also been cloned. The human ERα gene is located on chromosome 6 and the ERβ gene is on chromosome 14, demonstrating that they are in fact encoded by separate genes and are distinct [27]. A variety of ERβ mRNA isoforms have been described in humans, primates, rats and mice [29], but the 530 amino acid form of ERβ [28] is considered to be the wild type, full length human ERβ. Because the functional significance and expression of the various ERβ isoforms are unclear, the reader is referred to recent reviews [29,30].

Human ERα and ERβ (long form) share common structural domains, which are designated AF (Fig. 1). The A/B
domain contains activation function 1 (AF1), a constitutive activation function contributing to the transcriptional activity of the ER. This domain is one of the least conserved domains between ERα and ERβ, exhibiting only a 30% identity. Based on functional studies, ERβ has been shown to lack AF1 activity [31]. The DNA binding domain, or C domain, is the most highly conserved region between ERα and ERβ, with 96% identity. This allows both receptors to bind to similar target sites. The D domain, or hinge region, is not well conserved (30%) between the receptors and it contains the nuclear localization signal. Finally, the E/F region encompasses the ligand binding domain (LBD), a coregulator binding surface, the dimerization domain, a second nuclear localization signal, and activation function 2 (AF2). In contrast to AF1, AF2 is a ligand-dependent activation function. The E/F domains of ERα and ERβ exhibit a sequence identity of 53%.

Despite the 53% sequence identity between ERα and ERβ in the ligand binding domain, the two receptors exhibit subtle differences in ligand binding specificity. A \( K_D \) of 0.6 nM for ERα and 0.24 nM for ERβ for the ligand 16α-iodo-E2 was determined using saturation binding assays, which is similar to the range of E2 binding to ERs (0.1–1 nM) in various systems [32]. Additional studies using E2 showed that the \( K_D \) for ERα was 0.05 nM and for ERβ was 0.07 nM [33]. Despite the slight differences in the

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative binding affinity*</th>
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<tr>
<td>ERα</td>
<td>100</td>
</tr>
<tr>
<td>4-OHT</td>
<td>178</td>
</tr>
<tr>
<td>ICI164,384</td>
<td>85</td>
</tr>
<tr>
<td>DES</td>
<td>468</td>
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<tr>
<td>Genistein</td>
<td>5</td>
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* The relative binding affinity was calculated as a ratio of concentrations of E2 or competitor required to reduce the specific radioligand binding by 50%. Adapted from [32].
and a partial agonist where AF1 is dominant. ER activity so it is an antagonist in cells where AF2 is dominant therefore have the ability to distinguish between different is mediated by AF2, since ER ligands interact with ER subtypes in various parts of the human body (Fig. 2). The abundance and distribution of the receptors will, in part, determine whether a ligand and will have a particular effect. Using RT-PCR, Northern blot analysis, immunohistochemistry and in situ hybridization techniques, ERs and ERβ are known to be localized in the breast, brain, cardiovascular system, urogenital tract and bone[27,32,34,35]. ERs is the main ER subtype in the liver, whereas ERβ is the main ER in the colon. ERs and ERβ may also localize to distinct cellular subtypes within each tissue. For example, within the ovary, ERα is largely present in the thecal and interstitial cells, whereas ERβ is predominantly in the granulosa cells[36,37]. In the prostate, ERα localizes to the epithelium, whereas ERs localizes to the stroma[38].

3. Transcriptional activity

The transcriptional activity of the ER is mediated by AF1 and AF2 (Fig. 1) [39–42] and these regions were largely delineated using mutational studies. The activity of AF1 and AF2 differs depending on the cellular environment and promoter context[43]. In some cells, either AF1 or AF2 is dominant, and in others, both activation functions synergize [44]. In addition, AF1 and AF2 are differentially regulated by ligand. E2 is an agonist regardless of whether AF1 or AF2 is dominant. The pure antiestrogen ICI 164,384 blocks p68 binds CBP, so p68 may serve as a bridge to associate AF1 with AF2 coactivators. p68 enhanced the transcriptional affinity of ERs and ERβ for E2, both receptors are considered to have a similar affinity for E2. However, differences were observed for other ligands, such as antitestrogens and phytoestrogens.

A wide variety of structurally distinct compounds bind to the ER with differing affinities. Certain ligands act as ER agonists, and these include the natural ligand E2, as well as the synthetic estrogen diethylstilbestrol (DES). Certain phytoestrogens, which are environmental compounds produced by plants, can also be estrogenic. Genistein, present in soya beans and soy products, is a widely utilized phytoestrogen. Other compounds, such as ICI 182,780, are receptor antagonists. A final group of mixed agonists and antagonists are comprised of the SERMs and examples include tamoxifen and raloxifene. The relative binding affinity (Table 1) of ERs for various ligands are: DES (468) > 4-OHT(178) > E2(100) > ICI164,384(85) > genistein(5). In contrast, ERβ displayed the following relative binding affinities: 4-OHT(339) > DES(295) > ICI164,384(166) > E2(100) > genistein (36) [32]. These differences could result in functional consequences for a receptor subtype.

ER ligands interact with ER subtypes in various parts of the human body (Fig. 2). The abundance and distribution of the receptors will, in part, determine whether a ligand and will have a particular effect. Using RT-PCR, Northern blot analysis, immunohistochemistry and in situ hybridization techniques, ERs and ERβ are known to be localized in the breast, brain, cardiovascular system, urogenital tract and bone[27,32,34,35]. ERs is the main ER subtype in the liver, whereas ERβ is the main ER in the colon. ERs and ERβ may also localize to distinct cellular subtypes within each tissue. For example, within the ovary, ERα is largely present in the thecal and interstitial cells, whereas ERβ is predominantly in the granulosa cells[36,37]. In the prostate, ERα localizes to the epithelium, whereas ERs localizes to the stroma[38].

3.1. Coregulators

The initiation of transcription is complex and requires the interaction of many proteins at a target gene promoter. Transcriptional activation by the ER requires the recruitment of transcriptional regulators, such as general transcription factors, coactivators, corepressors, integrators, histone acetyltransferases, and histone deacetylases (reviewed in[47–49]). All of these regulators interact to affect transcription and the accessibility of target gene promoters.

Coactivators are proteins that enhance transcription. The contact between coactivators and the ER is made through the LXXLL motif present in the coactivator[50], although the site on the ER required for this interaction varies. Coactivators include steroid receptor coactivator 1 (SRC-1), SRC-2 and SRC-3, which are members of the p160 family. p300 and CREB-binding protein (CBP) are coactivators, in that they do not themselves bind DNA, but are recruited to promoters by other transcription factors, such as SRC-1. Corepressors decrease transcription and include nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT).

Local chromatin remodeling is remodeled to allow for gene transcription[47–49]. Chromatin remodeling factors include ATP-dependent nucleosome remodeling complexes and proteins that contain acetyltransferase activity. Histone acetylation correlates with transcription, whereas deacetylation correlates with gene repression. p300/CBP-associated factor (PCAF), p300/CBP, SRC-1 and SRC-3 contain intrinsic acetyltransferase activity. In contrast, corepressors do not contain histone deacetylase (HDAC) activity, but they recruit other proteins that have HDAC activity.

Although the majority of coregulators interact with multiple members of the nuclear receptor family, there are examples of coregulators that interact exclusively with the ER. In a screen to identify proteins that interact with the E/F domain of the dominant negative L540Q mutant ER, the protein repressor of estrogen receptor activity (REA) was isolated [51]. REA is a selective ERs and ERβ coregulator that enhances the inhibitory effectiveness of the L540Q ER and of antiestrogens. In contrast to other corepressors that interact with the unliganded ER, REA preferentially interacts with the liganded ER. In addition, REA binds to the L540Q mutant ER and antiestrogen-ligated ER more than the wild type ER. REA also competes with the coactivator SRC-1 for modulation of ER transcriptional activity. These studies suggest that when cellular REA levels are high, antiestrogen action will be enhanced [52].

Coactivators can also interact preferentially with a particular activation function region. For example, p68 RNA helicase is a coactivator specific for the ERs AF1 region [53]. p68 binds CBP, so p68 may serve as a bridge to associate AF1 with AF2 coactivators. p68 enhances the transcriptional
activity of the 4-OHT-ERα complex and the phosphorylation of S118 of ERα was required for the ability of p68 to enhance transcription.

In addition to interacting with both ERα and ERβ or a particular activation function, coactivators can interact selectively with ERα or ERβ. For example, SRC-3 enhances ERα and progesterone receptor (PR) stimulated transcription, but has no effect on ERβ-mediated transcription [54].

Therefore, coregulators provide an additional layer of specificity and regulation to the transcriptional activity of the ER. In addition to being a general ER coregulator, this could be accomplished by targeting ERα or ERβ specifically, or interacting with AF1 or AF2. The discovery of new coregulators will continue to define the selective role of either ERα or ERβ.

3.2. Ligands: E2, antiestrogens, phytoestrogens and subtype-specific ligands

Initial transcriptional activation studies using E2 were performed using ERβ cloned from rat prostate. These studies showed that an ERα-luciferase reporter was activated in the presence of E2 in CHO cells transfected with ERβ [24]. Studies in HepG2 and Hela cells showed that both AF1 and AF2 contribute to the activity of ERα, but the individual contributions depend on the cell context [31]. In contrast, AF2 mediates the transcriptional activity of ERβ to E2, since AF1 is inactive and inhibits the transcriptional activity mediated by AF2. In the presence of sub-saturating amounts of E2, ERα activity was actually suppressed by ERβ, suggesting that the relative amounts of ERα and ERβ can affect E2 activity [31].

The agonist activity of the antiestrogen tamoxifen has been shown to be dependent on cell type, promoter context, and ER subtype [55]. The agonist activity of tamoxifen appears to be mediated through ERα, because no agonism is observed with ERβ [31,55]. In fact, the addition of ERβ to ERα and tamoxifen inhibited the agonist activity of tamoxifen [31]. The lack of tamoxifen agonism at ERβ is likely to result from differences in the A/B region, and AF1 in particular, between ERα and ERβ.

Replacing the A/B domain of ERβ with the A/B domain of ERα/ERβ increased the transcriptional activity of the chimera in response to E2 compared to that observed with ERβ alone [56]. In addition, the ERα/β chimera displayed a transcriptional response to tamoxifen, which was not observed with ERβ. These studies further suggested the importance of ERα/A1 in mediating the agonist effect of tamoxifen. Therefore, differences in the A/B region between ERα and ERβ are responsible for the cell, promoter and ligand specificity displayed by the estrogen receptors.

Phytoestrogens are plant-derived compounds that are consumed in the diet. They contain inherent estrogenic activity or are converted to estrogenic compounds by bacteria in the gut. The first class of phytoestrogens is the isoflavonoids, which are present in soybean products, some fruits and vegetables, and red clover. Genistein, daidzein and glycitein are the main dietary-derived isoflavones [57]. Many of these compounds have a greater affinity for one ER subtype. For example, the isoflavone genistein has a 20-fold greater binding affinity for ERβ compared to ERα, but it activates transcription through ERα and ERβ [33]. The second class of phytoestrogens is the lignans, such as enterodiol and enterolactone, which are present in whole grain cereals, seeds, berries and nuts [58].

Interest in phytoestrogens and their potential role in the prevention of breast, prostate and colon cancer is a result of a higher incidence of these cancers in the western world compared to Asian populations [59]. The Asian diet is largely vegetarian or semivegetarian, which has a higher proportion of phytoestrogens compared to western diets that include more animal protein and fat. In general, phytoestrogens may provide some protection against breast, prostate and colon cancer (reviewed in [57,58]), but more human studies are needed before a definitive conclusion can be drawn.

All of the ER ligands described to date can bind to both ERα and ERβ, but with differing affinities. The development of agonists and antagonists that are selective for either ERα or ERβ would be useful tools to analyze the individual role of ERα or ERβ. For example, the R.R-enantiomer of 5,11-cis-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC) acts as an ERα agonist and an ERβ antagonist and has a sixfold greater affinity for ERβ over ERα [60]. Another example is methyl-piperidino-pyrazole (MPP), a basic side chain pyrazole that has a 200-fold binding selectivity for ERα over ERβ and is an ERα selective antagonist [61]. The structure function relationships of ERα and ERβ-specific ligands are reviewed in detail elsewhere [1,2].

3.3. Insight into the molecular basis for ER agonism and antagonism from crystal structures

The crystal structures of ligands complexed with the ER have provided invaluable insight into the structural basis of receptor agonism and antagonism (Fig. 3). The first studies of the human ERα LBD complexed with E2 and raloxifene indicated that although E2 and raloxifene bind at the same site within ERα, structural differences resulted. Helix 12 (Fig. 3A, green) is positioned over the ligand binding pocket in the ERα–E2 complex [62,63], thereby generating a functional AF2 that is able to interact with coactivators. In contrast, because the side chain of raloxifene is too long to fit within the binding pocket, it displaces helix 12 (Fig. 3C, compare arrows in 3A and 3C) [62,63]. This prevents the formation of a competent AF2 region. Subsequent studies directly analyzed the interaction of DES (Fig. 3B), 4-hydroxytamoxifen (4-OHT, the active metabolite of tamoxifen [64]) and the coactivator GRIP1 with human ERα [65]. Like raloxifene, the bulky side chain of 4-OHT displaces helix 12 (Fig. 3D, arrow) and places it in a position where it mimics bound coactivator.
The first structural description of ERβ was in complex with raloxifene or genistein [66]. The structure of the ERβ LBD was similar to the previously reported ERα LBD structure. In addition, the human ERβ-genistein (Fig. 3E) and the rat ERβ–raloxifene (Fig. 3G) complexes are quite similar. As in the ERα-4-OHT structure, the side chain of raloxifene displaces helix 12 and prevents it from sealing the ligand in the ligand binding pocket (Fig. 3G, arrow). In contrast, genistein binds to ERβ in a manner similar to E2. The main difference is that instead of helix 12 assuming the typical agonist position that E2 induces, helix 12 is in a more antagonist position (Fig. 3E, arrow). Genistein is a partial agonist at ERβ and coactivators must displace helix 12 into a more agonist conformation before activating transcription.

The only crystal structure of the pure antiestrogen ICI 164,384 with an estrogen receptor is with the rat ERβ LBD (Fig. 3F) [67]. The side chain of ICI 164,384 is longer than that of raloxifene. For the side chain of ICI 164,384 to achieve the same position that the side chain of raloxifene occupies, the steroidal core is flipped 180° around its longest (hydroxy-to-hydroxyl) axis. The position of the side chain exposes a large hydrophobic patch on the surface because the side chain of ICI 164,384 binds along the coactivator recruitment site. This abolishes the interaction of helix 12
with the LBD such that it is disordered. Therefore, helix 12 cannot move into position to form a coactivator recruitment site. This overall conformational change favors the recruitment of corepressors and resembles misfolded or denatured proteins, thereby targeting the ER for degradation.

In summary, when an agonist binds to the ER, a conformational change occurs that forms the AF2 coactivator binding site. However, the large side chain of antagonists such as tamoxifen, raloxifene, or ICI 164,384 protrudes from the binding cavity and displaces helix 12, thereby disrupting AF2. The molecular events resulting from ligand binding subsequently translate into agonism or antagonism at the ER (for review, see [63]). However, it is important to note that only the ER LBD has been used to generate these crystal structures, so the potential structural importance and interaction of the remaining part of the ER with the LBD has yet to be determined. In addition, a second binding site has been identified in the LBD of ER<sub>H9251</sub> and ER<sub>H9252</sub> [68]. Although multiple tetrahydrochrysene derivatives were docked into this second site, the classical steroid binding site was still the preferred site. Future studies could determine whether these multiple binding sites have consequences for the activity of the ER.

3.4. ER phosphorylation and ligand-independent transcriptional activity

In contrast to the ligand-mediated transcriptional activity or genomic effects described above, the activity of the ER can also involve ligand-independent or non-genomic effects. This is based on reports showing that many effects induced by E<sub>2</sub> occur within a short time frame of seconds to minutes, which is faster than transcriptional events. These rapid effects may be mediated in part by plasma membrane associated forms of the ER [69–71]. Interestingly, ERα and ERβ can both localize to the membrane [72].

The ligand-independent activity of the ER is a result of phosphorylation of the ER (Fig. 4) and creates cross-talk between the ER and other signaling pathways [73,74]. Phosphorylation of the ER is largely on serine residues in the AF1 region of ERα. The first reports showed that E<sub>2</sub> induced phosphorylation only on serine residues in MCF-7 cells [75]. In addition, using a transient transfection system in COS-1 cells, the ER was shown to be phosphorylated only on serine residues by E<sub>2</sub>, as well as other ligands such as 4-OHT and ICI 164,384 [76]. More specifically, E<sub>2</sub>, 4-OHT and ICI 164,384 induced ER phosphorylation at S118 [77]. Further evidence indicated that S118 was a major site of phosphorylation by E<sub>2</sub> and phorbol ester (TPA) in COS-1 cells [78] and that S118 is required for epidermal growth factor (EGF) activation of the ER via MAP kinase [79]. However, controversy exists as to which kinase phosphorylates S118 and the possibility exists that multiple kinases carry out this phosphorylation. S118 is a target of MAP kinase [80]. Other reports have shown that MAP kinase does not phosphorylate S118 in MCF-7 cells in response to E<sub>2</sub>, but EGF and PMA treatment result in phosphorylation of S118 via MAP kinase [81]. Further information suggests that MAP kinase phosphorylates S118 independent of ligand, whereas Cdk7 phosphorylates S118 in response to E<sub>2</sub> in COS-1 cells [82].

Serine residues in ERα other than S118 are also phosphorylated. In response to E<sub>2</sub>, S167 is the major phosphorylation site in recombinant ER expressed in Sf9 insect cells and MCF-7 cells and it is phosphorylated by casein kinase II [83]. Upon EGF or phorbol myristate acetate stimulation, S167 is a target of p90<sup>RSK1</sup>, which is phosphorylated by MAP kinase [84]. Phosphorylation of S167 has also been implicated in the phosphatidylinositol-3-OH kinase (PI(3)K)/Akt pathway. E<sub>2</sub> stimulates ERα binding to the p85<sub>α</sub> subunit of PI(3)K in endothelial cells, whereas ERβ does not exhibit this interaction with PI(3)K [85]. However, in HEK293 and MCF-7 cells, ERα is constitutively associated with p85<sub>α</sub> because this interaction is not affected by E<sub>2</sub> [86]. The end result is that Akt phosphorylates S167 of ERα and the consensus site is not present in ERβ [87].

The phosphorylation of S104 and S106 is mediated by the cyclin A-CDK2 complex in U-2 OS human osteosarcoma
cells [88]. S236 in the DNA binding domain is phosphorylated by protein kinase A and this phosphorylation regulates dimerization [89].

Tyrosine phosphorylation has been detected at Y537 in MCF-7 and S19 cells [90] and p65\textsuperscript{350-356} and p56\textsuperscript{354-360} mediate this phosphorylation event. Y537 is not phosphorylated by E\textsubscript{2} treatment, indicating that it is a basal phosphorylation site.

Although it is clear that many studies have focused on the role of phosphorylation of E\textsubscript{R\alpha}, few have studied E\textsubscript{R\beta}. The role of phosphorylation in the interaction between E\textsubscript{R\beta} and coactivators indicated that the interaction between mouse E\textsubscript{R\beta} and SRC-1 increased in the presence of E\textsubscript{2} [25]. Phosphorylation of the unliganded receptor by MAP kinase at S106 and S124 (S118 in human) in AF1 enhanced the recruitment of SRC-1 and therefore the activity of E\textsubscript{R\beta} [91]. The role of phosphorylation in the activity of E\textsubscript{R\beta} remains to be elucidated.

Activation of E\textsubscript{R\alpha} via phosphorylation at multiple sites (S104, S106, S118, S167, S236, Y537) by multiple kinases is important because of the interaction between growth factor signaling and the ER. Increased growth factor signaling may account for the loss of E\textsubscript{2} dependence, thereby producing antiestrogen resistant tumors. In addition, an association has been observed between elevated MAP kinase phosphorylation/activity and a poor response to endocrine therapy in breast cancer patients [92]. Although the precise relationship between ER phosphorylation and clinical outcome remains to be elucidated, the ER phosphorylation state has the potential to be a predictive biomarker and intervention target.

### 3.5. Non-classical pathways

The classical scheme of ER action involves ligand binding to the ER, dissociation of heat shock proteins from the ER\textsubscript{L/H9251} and SRC-1 increased in the presence of E\textsubscript{2} [25]. Phosphorylation of the unliganded receptor by MAP kinase at S106 and S124 (S118 in human) in AF1 enhanced the recruitment of SRC-1 and therefore the activity of E\textsubscript{R\beta} [91].

Activation of E\textsubscript{R\beta} via phosphorylation at multiple sites (S104, S106, S118, S167, S236, Y537) by multiple kinases is important because of the interaction between growth factor signaling and the ER. Increased growth factor signaling may account for the loss of E\textsubscript{2} dependence, thereby producing antiestrogen resistant tumors. In addition, an association has been observed between elevated MAP kinase phosphorylation/activity and a poor response to endocrine therapy in breast cancer patients [92]. Although the precise relationship between ER phosphorylation and clinical outcome remains to be elucidated, the ER phosphorylation state has the potential to be a predictive biomarker and intervention target.

### 3.5. Non-classical pathways

The classical scheme of ER action involves ligand binding to the ER, dissociation of heat shock proteins from the ER and receptor dimerization. The ER dimer then interacts with coactivators such as SRC-1 that are located in the regulatory regions of responsive target genes, and transcription is activated.

However, the ER can also mediate transcription via tethered interactions through protein-protein interactions at AF1 (reviewed in [93]) and Sp1 sites. In Hela cells transfected with E\textsubscript{R\alpha} and an AP1 reporter, E\textsubscript{2}, DES, raloxifene, tamoxifen, and ICI 164,384 stimulated reporter activity to varying degrees [94]. The amount of stimulation varied depending on the cell type [95]. In contrast, E\textsubscript{R\beta} activated the AP1 reporter in the presence of raloxifene, tamoxifen and ICI 164,384, but not with E\textsubscript{2} and DES. E\textsubscript{R\alpha} and E\textsubscript{R\beta} therefore respond differently to estrogens and antiestrogens at AF1 sites. The regions of the ER that are required for the stimulation of AP1-mediated transcription vary depending on the cell type and ligand [95-97].

The ER also activates transcription of target genes through ER-Sp1 protein interactions at GC-rich promoter elements. E\textsubscript{2}-responsive genes that activate transcription through non-consensus ERE half sites and GC-rich motifs include c-myc, creatine kinase B, cathepsin D, heat shock protein 27 and transforming growth factor \(\alpha\) [98]. E\textsubscript{R\alpha} and E\textsubscript{R\beta} have been shown to bind to the C-terminal domain of the Sp1 protein [99]. Transient transfections of MCF-7, Hela and MDA-MB-231 cells with a Sp1 reporter and E\textsubscript{R\alpha} or E\textsubscript{R\beta} showed varying patterns of activation by estrogen and antagonists. In MCF-7 and MDA-MB-231 cells, E\textsubscript{2}, 4-OHT and ICI 182,780 activated Sp1 through E\textsubscript{R\alpha}. In contrast, no changes were observed in Hela cells with any ligand. 4-OHT activated Sp1 through E\textsubscript{R\beta} in MCF-7 cells, but no changes were observed by any ligand in MDA-MB-231 cells [99].

All of the ligands decreased Sp1 reporter activity in Hela cells in the presence of E\textsubscript{R\beta}. Additional results [99] suggested that the relative amounts of E\textsubscript{R\alpha} and E\textsubscript{R\beta} present in a cell influence Sp1 activity. Amino acids 79–117 in AF1 are important for the interaction of E\textsubscript{R\alpha} with Sp1, and this region could mediate an association with other proteins that are important for the E\textsubscript{R\alpha}/Sp1 mechanism.

In summary, cell-specific regulation occurs as a result of multiple factors, including coactivator expression and recruitment, the ratio of E\textsubscript{R\alpha} to E\textsubscript{R\beta}, the nature of the ligand, ER phosphorylation and the pathway activated (ERE, Sp1 or AP1). The activity of E\textsubscript{R\alpha} and E\textsubscript{R\beta} is also complicated by the fact that they can form functional homo and heterodimers [28,31,100,101], which may activate different target genes. The inherent structural differences in the A/B domain between E\textsubscript{R\alpha} and E\textsubscript{R\beta}, where E\textsubscript{R\beta} lacks a functional AF1 domain, may result in a large effect on the activation profiles of target genes, especially when coactivators that interact preferentially with the AF1 domain are considered.

### 4. Normal physiological roles of E\textsubscript{R\alpha} and E\textsubscript{R\beta}

#### 4.1. Tissue distribution

Analysis of the tissue distribution of E\textsubscript{R\alpha} and E\textsubscript{R\beta} provides insight into the potential for targeting specific tissues. The relative distribution of E\textsubscript{R\alpha} and E\textsubscript{R\beta} mRNA was initially determined in rat tissues using RT-PCR [32]. E\textsubscript{R\alpha} mRNA was highly expressed in epididymis, testis, pituitary gland, ovary, uterus, kidney and adrenal. Moderate amounts were also present in the prostate gland, bladder, liver, thymus and heart. Highest amounts of E\textsubscript{R\beta} mRNA were detected in the prostate gland and ovary. In the rat ovary, E\textsubscript{R\beta} is the predominant ER in the granulosa cells, whereas E\textsubscript{R\alpha} is largely present in the thecal and interstitial cells [36,37]. Uterus, bladder, lung and testis showed intermediate levels of E\textsubscript{R\beta}, whereas low but detectable levels of E\textsubscript{R\beta} were observed in epididymis, the pituitary gland, thymus, various brain sections and spinal cord. Target tissues with higher expression levels of E\textsubscript{R\alpha} are predicted to be more affected by ER ligands. The expression in rat is similar to that observed in humans (see Section 2) [135] and references therein).
4.2. Knockout mouse studies

Transgenic mice are valuable experimental models to elucidate gene functions. Knockout mice, where genes of interest have been deleted, provide a basic insight into the normal functions of genes during development and at maturity. The classical scheme of reproductive organ development is that female reproductive tract development is the default pathway and that estrogens are not required for the initial differentiation and development of the female reproductive system. In contrast, testosterone is essential for the proper development of male structures. The potential role of estrogen in male development has remained unclear. Knockout mouse models have been utilized to analyze the role of ERs and ERβ in the general development and physiology of the mouse (Table 2). These models include ERα knockout mice (ERKO), ERβ knockout mice (βERKO) and both ERs and ERβ knockout mice (αβERKO) (reviewed in [102–105]). It is interesting to note that a loss of either of ERs and/or ERβ is not lethal, and the mice survive to adulthood.

4.2.1. Reproductive phenotypes in ERα knockout mice

ERα knockout mice show no abnormal external phenotypes. However, the most striking phenotypes occur in the tissues that predominantly express ERα, such as the uterus and mammary gland, but defects are also observed in the ovary and in sexual behavior. One role of the ovary is to act as an endocrine organ by providing sex steroids to the female and is essential for proper reproductive functions. The ovaries in αERKO mice contained cystic and hemorrhagic follicles that contain no corpora lutea and few granulosa cells [106]. Adult females have hypoplastic uteri and showed no responses to estrogen, such as increases in uterine wet weight, hyperemia, or the alteration of vaginal epithelial cell morphology [106]. Females exhibit little sexual behavior in that they display no lordosis posture or receptivity, indicating a lack of estrogen responsiveness in the central nervous system [106]. As a result of these phenotypes, female mice lacking ERα are infertile.

The mammary glands of the adult αERKO look essentially like those of a female mouse before puberty, indicating that rudimentary mammary glands can develop independent of estrogen and ERα, but a fully differentiated gland requires ERα [107]. αERKO mice were further used to study the potential protective effect of dietary genistein on mammary tumor development. The rationale for this study is the suggestion that genistein could be protective against breast tumors. Because genistein has a greater binding affinity for ERβ when compared to ERα, genistein is predicted to act through an ERβ pathway. αERKO mice or mice containing wild type ERα were fed genistein and treated with dimethylbenz[a]anthracene. Tumors formed in the mice with wild type ERα, whereas no tumors were present in the αERKO mice [108]. This indicated that genistein did not provide protection against tumor formation in the wild type mice and that it could actually result in tumor formation in situations where ERβ is dominant, such as in the αERKO mice, ERβ (genistein) is protective against breast tumors, but when ERα is present, such as in the wild type mice, ERβ (genistein) is no longer protective. These data, combined with the high expression levels of ERs in the mammary gland, point toward ERs being the main mediator of estrogen action in the mammary gland.

Table 2

<table>
<thead>
<tr>
<th>Phenotypes of ER knockout mice</th>
<th>αβERKO no ERα, ERβ dominant</th>
<th>βERKO no ERβ, ERα dominant</th>
<th>αβERKO no ERα or ERβ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reproductive tract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Infertile</td>
<td>Reduced fertility</td>
<td>Infertile</td>
</tr>
<tr>
<td>Male</td>
<td>Hypoplastic uterus; cystic and hemorrhagic follicles; no corpora lutea; few granulosa cells in ovary</td>
<td>Low sperm count; low sperm motility; low testis weight</td>
<td>Hypoplastic uterus; develop structures similar to male seminiferous tubules (sex reversal) in ovary</td>
</tr>
<tr>
<td><strong>Mammary glands</strong></td>
<td></td>
<td>Reduced fertility</td>
<td>Infertile</td>
</tr>
<tr>
<td>Female</td>
<td>N/A</td>
<td>Normal uterus; many early atretic follicles; Fewer corpora lutea in ovary</td>
<td>N/A</td>
</tr>
<tr>
<td>Male</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Sexual behavior</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>No lordosis; no receptivity</td>
<td>Normal mounts; reduced intro-missions; rarely ejaculates</td>
<td>No mounts, intro-missions or ejaculation</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td><strong>Bone</strong></td>
<td></td>
<td>Germ cell density, diameter and length</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Decreased density, diameter and length</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Decreased density, diameter and length</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

N/A: not applicable.
Male oERKO mice show decreased sperm motility [109], a low sperm count, and low testis weight, resulting in reduced male fertility [106]. In terms of sexual behavior, male oERKO mice show normal levels of mounts, but also reduced intromissions, and rare ejaculations [110], which also contribute to the reduction in fertility.

4.2.2. Reproductive phenotypes in ERβ knockout mice

ERβ knockout mice have also provided additional information as to the function of ERs in the mouse. The most obvious phenotype occurs in the ovary, which is a tissue that contains the greatest expression of ERβ. Ovaries in females lacking ERβ have more early atretic follicles and fewer corpora lutea when compared to wild type females [111]. Further experiments indicate a partial arrest of follicular development and a decrease in the frequency of follicular maturation [111]. These female mice show normal sexual behavior [112] and normal mammary gland structure. In addition, ERβ knockout females have fewer litters and fewer pups per litter. Therefore, ERβ knockout mice show reduced fertility.

In contrast to ERα knockout females, ERβ knockout males are fertile and show normal sexual behavior [112]. However, older males show epithelial hyperplasia in the collecting duct of the prostate and the bladder wall [111]. jERKo mice were developed by another group that exhibited many of the same phenotypes, except that no prostate or bladder hyperplasia was observed [113]. The reason for these differences is unknown. jERKo mice also display abnormalities in the brain, such as regional neuronal hypocellularity, which progressed to a degeneration of neuronal cell bodies with age [114].

4.2.3. Reproductive phenotypes in ERα/ERβ knockout mice

To create a complete picture of ER function in male and female development, mice with both ERα and ERβ knockouts (oERKO) were developed. As with the ERα and ERβ single knockouts, oERKO mice survive to adulthood and exhibit no abnormal external phenotypes [115].

Young oERKO females show proper differentiation of the uterus, vagina and cervix. However, once the mice reach 2.5–7 months of age, uterine hypoplasia is observed. Because this phenomenon is observed in both oERKO and oERKO mice, it is a hallmark of ERα loss in the uterus. The most interesting phenotype occurs in the adult oERKO ovary, where structures reminiscent of male seminiferous tubules of the tests were observed [115]. These structures were not present in the prepubertal ovaries. This is the first example of sex reversal in an adult mouse gonad because the female ovarian cells are able to re-differentiate to a male Sertoli cell phenotype.

oERKO males are infertile and show an 80% reduction in epididymal sperm number and a 5% decrease in sperm motility [115]. They also show no components of sexual behaviors [116].

4.2.4. Bone density and cardioprotection

An established association exists between the declining levels of estrogen at menopause and the development of osteoporosis, implicating estrogen in the maintenance of bone mass. oERKO females and males showed decreases in femoral length and diameter as well as density [105]. jERKo mice had normal bone length and density [102], emphasizing the important role for ERs in bone.

Cardiovascular disease in women increases after menopause. As a result of the suggestion that estrogen might be cardioprotective, there has been an interest in studying the role of the ERs in the cardiovascular system. Indeed, the publication of the negative results of the Women’s Health Initiative [16] has increased interest in this aspect of female physiology to discover mechanisms of physiological benefit that can be separated from disadvantageous side effects such as fatalities from cardiovascular complications.

Models of carotid injury have been developed in knock-out mice to study the individual contribution of ERα and ERβ in cardioprotection. In wild type and oERKO mice, E2 treatment inhibited the increase in carotid medial vessel wall area and vascular smooth muscle cell proliferation normally observed in injured carotid arteries [117]. Because E2 inhibits markers of vascular injury in the oERKO mice, ERα is clearly not required for this process. Similar results were observed in the jERKo mice [118], suggesting that ERα and ERβ are individually redundant in mediating the vasoprotective effects of E2. In contrast, E2 did not protect against the increase in carotid medial wall area after injury in oERKO mice, but did inhibit vascular smooth muscle cell proliferation [119]. Unfortunately, the specific batch of oERKO mice exhibited uterine weight increases in the presence of E2, suggesting residual ERα activity. In fact, it has been suggested that the original oERKO mice [106] express a smaller ER transcript [120] that may have some functional activity [119,121]. As a result, these data [119] should be interpreted with caution. Nevertheless, overall studies in ERα or ERβ knockout mice suggest that either ERα or ERβ can mediate the E2-induced protection of vascular injury.

It is important to consider whether E2 also has a role in maintaining the vasculature by mediating increases in nitric oxide synthase, which ultimately results in vasodilation. Wild type male mice have increased basal levels of endothelial nitric oxide in the aorta when compared with male oERKO mice [122]. oERKO mice also display increases in L-type Ca2+ channels in the heart, which could lead to abnormalities in cardiac excitability [123]. These studies suggest that ERs is involved in cardiac modulation through the regulation of nitric oxide synthesis as well as cardiac Ca2+ channel expression.

4.3. Summary of findings with ER knockouts

Overall, these transgenic mice studies provide insight into the role of estrogen receptors in the development and function of reproductive structures. In the case of the male, ERs...
can also be targeted directly using SERMs such as tamoxifen and raloxifene as competitive inhibitors of estrogen action, or by the removal and degradation of the ER by pure antiestrogens such as ICI 182,780 (fulvestrant).

Endocrine manipulations are among the least toxic and most effective therapies for the treatment of hormone-responsive breast cancers. In the clinic, factors such as ER, PR and nodal status have historically predicted response to endocrine therapy (Table 3). Patients that are ER+ show a 53% objective response rate to endocrine therapy, and this can be divided into 69% for ER+PR+ and 32% for ER+PR− [128]. As expected, 13% of ER− patients respond to therapy, with 32% of the ER−PR− and 11% of the ER−PR+ group exhibiting a response. 66% of patients with PR+ tumors will respond, versus 22% with PR− tumors. The measurement of receptor status has changed from the ligand binding assay to immunohistochemical methods. Nodal positivity is another predictor of response to endocrine therapy. Tumors that contain positive nodes correlate with a lower disease free survival (DFS) [129]. If a tumor population is node positive or node negative, the presence of the ER correlates with better DFS. Current treatment strategies have shown that 5 years of adjuvant tamoxifen treatment is beneficial in pre- and postmenopausal women with ER-positive tumors [130]. In addition, tamoxifen can be used for the prevention of breast cancer [131].

5. The role of ERs and ERβ in cancer

The analysis of knockout mice has provided a framework in which to study the potential functions of ERα and ERβ in human target tissues. Phenotypes of oERKO mice have pointed toward the importance of ERs in the uterus and mammary gland of females. In addition, βERKO mice have suggested an important function for ERβ in the ovary in females and in the prostate gland in males. The laboratory studies in mice naturally advance the study of the complex role of the individual ERs in human cancer.

5.1. Breast cancer

5.1.1. ERα and breast cancer

The ER is an important target to develop drugs for the treatment and prevention of breast cancer [127]. The interaction of estrogen with the ER can result in increased proliferation of target cells so the rationale for endocrine therapy is to block the interaction of estrogen with the ER. This goal can be accomplished by blocking the production of estrogen by ovarectomy, or inhibiting the conversion of steroidal precursors to estrogen using aromatase inhibitors. The ER can also be targeted directly using SERMs such as tamoxifen and raloxifene as competitive inhibitors of estrogen action, or by the removal and degradation of the ER by pure antiestrogens such as ICI 182,780 (fulvestrant).
breast cancer cell lines where tamoxifen is an antagonist, tamoxifen recruits corepressors; however, in endometrial cells where tamoxifen is an agonist, tamoxifen recruits coactivators [134]. SRC-1 is necessary for the agonist activity of tamoxifen in endometrial cells [134]. These findings suggest that differences in coregulator recruitment to a promoter can determine the functionality of the ER in different tissues.

Changes in coregulator levels affect target gene expression and might change the gene activation profile to one supporting a proliferative phenotype. This molecular mosaic appears to correlate with clinical outcomes. Studies analyzing SRC-1 and tamoxifen showed that high SRC-1 levels may correlate with a favorable response to tamoxifen treatment in women with recurrent breast cancer [135]. Gene amplification and overexpression of “amplified in breast cancer-1” (AIB1/SRC-3) has been documented in breast and ovarian cancer cell lines and breast cancer biopsies [136,137]. Patients with ER-positive breast tumors expressing high levels of SRC-3 and HER2 display a poor outcome with tamoxifen therapy [138]. In contrast, high SRC-3 levels are associated with better prognosis in patients not receiving adjuvant tamoxifen. The authors’ hypothesis is that increased HER2 signaling results in activation of ER-3 and the ER via phosphorylation, which results in tamoxifen resistance. Clearly, the strategic goal of establishing the importance of both ERs and ERβ in the outcomes of endocrine therapy should be addressed and resolved using the tissue resources of the ATAC trial of tamoxifen versus anastrozole [12].

5.1.2. Tamoxifen resistance

Despite over 30 years of clinical experience with tamoxifen, for most patients, tumors that initially regress with tamoxifen will eventually recur and require alternate treatment. The mechanisms of cellular resistance to tamoxifen are under investigation (reviewed in [139–142]).

Most resistant tumors continue to retain a functional ER [143], so loss of the ER is not sufficient to explain resistance. Because a functional ER is present, the cells have changed how the ER–tamoxifen complex is perceived and how it signals, or there is altered expression of genes to counteract tamoxifen signaling. During the development of resistance, tamoxifen could become an estrogen agonist by inducing E2-specific genes [144,145]. Tamoxifen resistance could also be explained by ER mutations, coregulator expression and recruitment, or interactions with other signaling pathways. Additionally, a number of nonspecific mechanisms may contribute to the tamoxifen-resistant phenotype. The mechanisms may include events that limit the intracellular availability of tamoxifen, such as binding to other proteins, partitioning into lipophilic membrane domains, altered transport into or out of the cell, the development of oxidative stress or the conversion of tamoxifen to other metabolites. Other mechanisms include overexpression of growth factors, increased angiogenesis or heterogeneity in the tumor cell population (reviewed in [146]).

Mutations in ERs have been demonstrated in breast tumors, but there is no reason to believe they play a major role in tamoxifen resistance at this point. Nevertheless, if these mutations develop during the course of tamoxifen therapy, the tumor cells could begin to respond differently to tamoxifen. The K303R mutation results in increased sensitivity to estrogen [147]; however, tamoxifen is still effective in blocking estrogen action. In addition, the Y537N mutation results in constitutive activity of the ER that is unaffected by E2, tamoxifen and ICI 164,384 [148]. The D351Y mutation was discovered in tamoxifen-stimulated MCF-7-derived breast tumor models [149]. Despite the fact that the mutation enhances the estrogen-like action of SERMs [150,151], there is no general enhancement of mutations in tamoxifen resistant disease [152–156]. Knowledge of the D351Y mutation in ERs and the close association of D351 with the antiestrogenic side chain of SERMs have provided an important insight into the molecular modulation of the SERM–ERs complex [12,157]. The balance between ERα and ERβ may also play a role. ERβ mRNA was shown to be upregulated in tamoxifen-resistant human tumors and cell lines, suggesting that ERβ is a poor prognostic factor for the development of drug resistance [158].

Since the target of SERM action is the ER, it is clear that increases or decreases in coregulatory molecules will modulate the SERM–ER complex to be estrogenic or antiestrogenic, respectively. This principle is illustrated by the finding of elevated levels of the coactivator SRC-3 in tumors that fail on tamoxifen, but only in the presence of cell surface signaling [138]. It is known that repression of gene activation by tamoxifen is an active process, where tamoxifen recruits corepressors [159]. If corepressor levels are low in a particular tumor, tamoxifen may be unable to recruit a sufficient amount of coactivators to silence gene transcription, thereby contributing to drug resistance.

Alterations in signal transduction pathways are another mechanism of tamoxifen resistance. Examples include resistance to the growth inhibitory effects of TGF-β [153], enhanced AP1 signaling [160–163] and upregulation of Akt/PI3K [86,87], HER2 [164–166], IGF-1 receptor [167], and active MAP kinase [168]. Activation of these pathways can lead to ligand-independent activation of the ER via phosphorylation.

Overall, there are numerous potential mechanisms that may contribute singly or in combination to the development of drug resistance. Despite the possibility of drug resistance, there are potential treatments after the development of tamoxifen resistance. These include the use of aromatase inhibitors to block the production of estrogen [12] as well as pure antiestrogens to degrade the ER [11].

5.1.3. ERβ and breast cancer

The role of ERβ in breast cancer growth and development is not as clear as the role of ERα (reviewed in [169,170]). ERβ might have a modulating effect in breast cancer because it is expressed in normal and malignant breast tissue,
whereas no evidence of hyperplasia was observed in the cancer therapy. ER antiproliferative role. If this scenario is proven correct, Secondly, one report analyzing ER susceptible to the effects of environmental estrogens [177]. gland expresses a large amount of ER normal and malignant prostate tissue. Because the prostate ER prostate, ER expression at high levels in the prostate [32]. Within the prostate, ER localizes primarily in the epithelium, whereas ERα is in the stroma [38]. ERβ has also been detected in normal and malignant prostate tissue. Because the prostate gland expresses a large amount of ERβ, it may be more susceptible to the effects of environmental estrogens [177]. Secondly, one report analyzing ERβ knockout mice showed that these mice displayed prostatic hyperplasia [111], whereas no evidence of hyperplasia was observed in the αERKO mice. This suggests that ERβ may protect against abnormal growth in the prostate. Although the role of ERβ in the prostate remains unclear, most findings support an antiproliferative role. If this scenario is proven correct, ERβ selective ligands could potentially be used for prostate cancer therapy.

5.3. Colon cancer

The distribution of ERα and ERβ has been evaluated in colon cancer cell lines as well as human colon cancer samples. ERβ is present in the human colon cancer cell lines HCT116, HCT8, DLD-1, LoVo, HT29, Colo205, SW480 and Colo205 but ERα is not present [178–180]. Studies on human samples showed that ERα protein was expressed at extremely low levels in normal and malignant colon tissue compared to ERβ levels [181]. Although no differences were observed in ERβ mRNA levels using RT-PCR between normal and malignant colon tissue, a loss of ERβ protein was observed during malignant transformation [181]. In addition, the localization of ERβ in normal colon was nuclear, whereas a cytoplasmic localization was also observed in colorectal carcinoma tissue [182]. Therefore, multiple studies support the idea that ERβ is the primary ER expressed in the colon, and that the loss and change in localization of ERβ is associated with the progression to cancer.

The recent results of the Women’s Health Initiative (WHI) trial have provided an interesting insight on the role of estrogen in colon carcinogenesis [16]. The primary goal of the WHI was to evaluate the use of hormone replacement therapy (HRT, estrogen plus medroxyprogesterone acetate) in postmenopausal women aged 50–79 for the prevention of cardiovascular disease. Other outcomes were also documented. The HRT group had a 26% increase in breast cancer rates, which was anticipated [17,183]. Colorectal cancer rates were reduced by 37%, which was also suggested in previous studies [184]. The decreased incidence of colon cancer could be mediated by ERβ. In women, ERβ mRNA levels have been shown to be decreased in colon tumors compared to normal tissue, whereas ERα levels did not change and are much lower than ERβ levels [185]. This evidence suggests that the activation of ERβ in the colon by HRT (estrogen) provides protection against colon cancer. This is similar to the situation observed in prostate cancer, where ERβ is thought to play a protective role.

5.4. Ovarian cancer

Although approximately two-thirds of ovarian cancers are ER positive, responses to endocrine therapy are modest [186]. However, contraceptives that combine estrogen and progestins decrease the risk of ovarian cancer, such that 5 years of contraceptive use confers a 50% risk reduction that persists for at least 10 years after the cessation of use [187]. ERβ is the predominant ER in the ovary, where it is found in the granulosa cells, whereas ERα localizes to the thecal and interstitial cells. Knockout mouse studies have shown that αERKO mice ovaries contained cystic and hemorrhagic follicles that contained no corpora lutea and few granulosa cells [106]. Ovaries in αERKO mice have more early atretic follicles, fewer corpora lutea, a partial arrest of follicular development and a decrease in the frequency of follicular maturation [111].
Studies of normal and malignant human ovaries have yielded conflicting results. One study showed an increase in ERα mRNA relative to ERβ in ovarian cancer compared to normal ovary [188]. Another study showed varying amounts of ERs in normal ovary, lower levels of ERβ in ovarian epithelial primary tumors, and only ERαs in metastatic tumors [189]. ERβ levels were lower than ERα levels in ovarian cancer compared to normal tissue [190]. In contrast, a decrease in ERs mRNA was observed relative to ERβ in human ovarian surface epithelial cells [191]. The majority of studies therefore support a scenario in which ERs becomes the dominant ER in ovarian cancer. This implies a mechanism that results in ERs overexpression or a selective advantage for ERs-positive cells. Further studies are needed to fully determine the contributions of ERα and ERβ to ovarian cancer.

Overall, ERβ appears to play a protective role against the development of breast, prostate and colon cancer. A variety of mechanisms supporting an antiproliferative role for ERβ have been proposed to explain these findings. In ERβ+/ERα− colon cancer cell lines, estrogen has no effect on cell growth, but genistein slightly inhibited cell growth [179]. In addition, transfections of the cDNA for ERβ inhibited the growth, invasion and motility of the MDA-MB-231 breast cancer cell line [192]. These data support a scenario in which activation of ERβ-mediated pathways is able to suppress cell growth. Another possibility is that the presence of ERβ could simply antagonize the growth stimulatory effects mediated by ERα. This is suggested by a study in which ERβ inhibited the agonist activity of ERs–tamoxifen complex [31]. Further studies are needed to dissect the precise interactions between ERα and ERβ in cell growth control.

6. Current status of the ER and future research directions

It is clear that ERα and ERβ are extremely important components of a complex signal transduction pathway that specifically regulates the growth and development of target tissues and tumors. At the molecular level, ERs act as transcription factors to target a variety of genes using the classical ERE pathway or tethering mechanisms utilizing AP1 or SP1. Usually, transcriptional activity is in response to endogenous ligands such as steroid estrogens or other ligands such as antioxidants or phytoestrogens. The transcriptional activation of the ER results in the activation of target genes that are involved in normal physiological processes such as the maintenance of bone density, proper reproductive organ development, fertility and behavior. Aberrant roles for ERs have been demonstrated in breast cancer and ERβ could be involved in prostate cancer and colon cancer.

Knowledge of the role of estrogen in physiology and pathology has resulted in the development of effective and relatively safe drugs that target endocrine-related breast cancer, postmenopausal osteoporosis and resulted in recent advances toward the prevention of breast cancer [1,2]. The developing molecular knowledge of estrogen action can be further exploited to design better drugs to target ERα and ERβ selectively. By way of a recent example, 2,3-bis(4-hydroxyphenyl) propionitrile (DPN), an ERβ selective ligand, has a 70-fold higher relative binding affinity and a 170-fold higher potency for ERβ over ERα in transcription assays [193]. Propyl pyrazole triol (PPT) is an ERα selective ligand, with a 400-fold affinity for ERα over ERβ [194]. PPT treated rats exhibited uterine weight gain, increased complement C3 mRNA in the uterus and increased progesterone receptor mRNA in the brain. Therefore, ERα and ERβ selective ligands could be utilized to delineate the specific physiological processes mediated by ERαs and ERβs. This knowledge can be further exploited to develop ER subtype-specific therapeutic drugs.

The application of subtype-specific drugs for therapeutic use requires consideration of ER subtype expression in target tissues. For example, the cellular environment of breast cancer is comprised of high levels of ERα and extremely low levels of ERβ. Because estrogen is a growth stimulus through ERα, the use of aromatase inhibitors or ERα-specific antagonists to block this interaction may be optimal. Since evidence [171–174] indicates that ERβ is protective in the breast, the combined use of an ERβ agonist could provide further benefit. A different scenario occurs in the prostate gland and colon, where ERβ is the dominant ER and little or no ERαs is present. ERβ is viewed as protective in these tissues, so the use of an ERβ agonist may provide the most benefit. However, the situation becomes more complicated in tissues that contain high levels of both ERαs and ERβs, because the interaction that will occur by activating or blocking both ERs separately must be deciphered.

The issue of receptor interaction is extremely difficult to address in tissues throughout the body. Nevertheless, one issue that has important physiological implications is whether even a small amount of ERβ can cause a significant response in the presence of a large amount of ERα. The question to be answered is how much of an ER is required to elicit a response. In other words, if a large amount of ERs is present, can the activation of even a small amount of ERβ cause a significant response through alternate signal transduction pathways? If future strategies to design ER subtype-specific drugs are pursued, then the physical chemistry of the agents will be extremely important. It is extremely difficult to control the pharmacokinetics and pharmacodynamics of orally active medicines, especially when the compliance of patients is extremely variable. Treatment strategies involve blocking the activation of a signal transduction pathway, but in the future, a specific ER agonist may be used that inadvertently is detrimental to some unknown target. Thus, the development of a selective ligand with a high affinity for one ER subtype may inadvertently interact with the other ER subtype in some patients if dosing is too high or drug interactions result in inappropriate accumulation.
Although the complexities of ERα and ERβ function in target tissues remain to be fully characterized, the knowledge gained to date has provided a solid foundation for further progress. Future discoveries could include the development of the perfect SERM. Additionally, a combination of ER subtype-specific ligands may be able to control cancer in specific target sites or improve current treatment strategies for a variety of debilitating diseases linked to estrogen withdrawal following menopause. Most importantly, the elucidation of the complex signal transduction pathways might open the door to novel therapeutic strategies not previously considered appropriately. Clearly, the close cooperation of laboratory science with clinical outcomes has enhanced our knowledge of the disease process in breast and endometrial cancer with established agents for treatment. The promise for the future is to target the ERs to prevent colon, prostate, and ovarian cancer. Indeed, this concept would not have appeared to be reasonable a decade ago and has resulted from advances in molecular biology in the laboratory.

References


Biographies

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