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Introduction

Estrogen receptors (ERs[1]) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. In the late 1950s, the existence of a receptor molecule that could bind 17β-estradiol was demonstrated by Jensen and Jacobsen (Jensen and Jordan, 2003). The first ER was cloned in 1986 (Green et al., 1986; Greene et al., 1986). This receptor was regarded as the only ER until a second ER was reported in 1996 (Kuiper et al., 1996). The two receptors are today known as ERα and ERβ, respectively. ERα and ERβ show a high degree of similarity when compared at the amino acid level. The amino acid sequence identity between ERα and ERβ is approximately 97% in the DNA-binding domain and approximately 56% in the ligand-binding domain (LBD), whereas the N terminus is poorly homologous at 24%. Transcriptional activation by ERα is mediated by two distinct activation functions: the constitutively active AF-1, located in the N-terminal domain of the receptor protein, and the ligand-dependent AF-2, located in the C-terminal domain of the receptor protein. ERβ seems to have a weaker corresponding AF-1 function and thus depends more on the ligand-dependent AF-2 for its transcriptional activation function (Delaunay et al., 2000). The relative importance of the AF-1 and AF-2 activation functions depends on cellular and promoter context (Tzukerman et al., 1994).

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[1] Abbreviations: ER, estrogen receptor; LBD, ligand-binding domain; CVD, cardiovascular disease; KO, knockout; SERM, selective estrogen receptor modulator; STAR, study of tamoxifen and raloxifene; THC, tetrahydrocannabinol.

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ER Expression

ERα and ERβ can be detected in a broad spectrum of tissues. In some organs, both receptor subtypes are expressed at similar levels, whereas in others, one or the other subtype predominates. In addition, both receptor subtypes may be present in the same tissue but in different cell types. ERα is mainly expressed in, for example, uterus, prostate (stroma), ovary (theca cells), testes (Leydig cells), epididymis, bone, breast, various regions of the brain, liver, and white adipose tissue. ERβ is expressed in, for example, colon, prostate (epithelium), testis, ovary (granulosa cells), bone marrow, salivary gland, vascular endothelium, and certain regions of the brain.

Mechanism of Transcriptional Activation

Like other steroid hormone receptors, ERs act as dimers to regulate transcriptional activation. Transcriptional control by ERs requires interaction with coregulator complexes, either coactivators for stimulation or corepressors for inhibition of target gene expression (Klinge, 2000). Although we have named a consensus estrogen response element (ERE; GGTCAnnnTGACC), most estrogen responsive genes do not contain this perfect palindromic consensus sequence. This includes well studied estrogen target genes such as pS2, cathepsin D, and progesterone receptor. In addition to binding to the classic ERE on DNA, the activated ERs can activate gene expression through DNA sequences that are primary targets for other transcription factors such as cAMP-responsive elements and GC-rich Sp-1-binding sites (Safe, 2001). In this case, ERs are thought to bind to DNA-bound AP-1 and Sp-1, respectively. Selective action of ERα and ERβ in vivo probably results from a complex interplay at a given time point between expression levels of each ER, the relative affinity for a specific response element, ligand and cofactor availability, and interaction with other transcription factors. A signifi-

cant number of post-translational modifications of ERs have also been described, including phosphorylation, acetylation, SUMOylation, and ubiquitination, which affect receptor activity and/or stability.

ERα and ERβ Promoters

A number of promoters have been described for ERα; some show tissue specific activation (Kos et al., 2001). For ERβ, two promoters, ON and OK, have thus far been characterized (Li et al., 2000). Methylation of the ERβ ON promoter has been shown to be inversely correlated with mRNA expression (Zhao et al., 2003). It is possible that additional, not yet identified promoters also regulate the expression of ERs.

Biologically Important Isoforms and Mutations

Genetic screening of the *ERα* gene locus has revealed the existence of several polymorphic sites (del Senno et al., 1992). The most widely studied are PvuII (T397C) and XbaI (C351G) restriction fragment-length polymorphisms in intron I and the $(TA)_n$ variable number of tandem repeats within the promoter region of the gene. In different studies, these polymorphisms have been associated with several pathological conditions such as breast and prostate cancer, osteoporosis, Alzheimer's disease, and cardiovascular diseases (CVDs) (Brandi et al., 1999; Dunning et al., 1999; Herrington et al., 2002). Similarly, several polymorphic sites have been identified for ERβ (Rosenkranz et al., 1998). ERβ polymorphisms have been shown to be associated with several conditions, including blood pressure, bone mineral density, and bulimia (Ogawa et al., 2000a,b; Nilsson et al., 2004).

ER A908G

The A908G (K303R) ERα somatic mutation was first identified in premalignant breast lesions (Fuqua et al., 2000) and seems to be a gain-of-function mutation, with activation at low concentrations of hormone and altered binding to ERα coactivators, which is associated with a hypersensitive phenotype. The mutation interrupts the major site of ERα acetylation (Wang et al., 2001) and creates an efficient protein kinase A phosphorylation site (Cui et al., 2004). Although the presence of the mutation is controversial because a number of investigators have failed to detect the mutation using fluorescent dideoxysequencing (Tokunaga et al., 2004), it has recently been reported in invasive tumors using more sensitive microsequencing techniques (Conway et al., 2005).

ERα46

ERα46 is the result of initiation at an internal ATG from alternatively spliced mRNAs where the exon encoding the normal ATG is spliced out. This protein lacks the first 173 amino acids of the ERα protein, including AF-1 (Flouriot et al., 2000). Consequently, ERα46 is active with regard to transcriptional activation in cells and tissues where the receptor mainly relies on AF-2 for activity. In cells and tissues where ERα AF-1 is critical for activity, ERα46 acts as an inhibitor of ERα function (Flouriot et al., 2000). ERα46 has been detected in several cell types, including osteoblasts and endothelial cells.

ERβcx

The human variant ERβcx has an alternative last exon and C terminus. The last 61 amino acids encoding parts of helix 11 and helix 12 are replaced by 26 novel amino acids. ERβcx does not bind any ligands tested; however, it might be possible to identify pharmacological ligands. When ERβcx is cotransfected with ERα or ERβ, it inhibits ligand-induced transactivation and DNA binding by ERα but does not influence ERβ-mediated transactivation and DNA binding (Ogawa et al., 1998). Interestingly, it has been shown that expression of ERβcx is increased in several forms of cancer, including cancer of the breast, ovary, and prostate (Fuqua et al., 1999). An ERβcx-like exon is not found in mouse and rat.

ERβins

ERβins (ERβ2) contains an extra 54-base pair insertion into the reading frame, causing an 18-amino acid insertion into the LBD between exons 5 and 6. This insertion occurs through alternative splicing. ERβins has been described for mouse and rat. In humans, the ERβins exon is out of frame with the rest of the coding sequence. ERβins generally shows impaired ligand binding and transcriptional activation (Chu and Fuller, 1997). ERβins acts as a dominant-negative regulator of ERβ and ERα and causes a dose-dependent inhibition of ERβ and ERα transcriptional activity (Maruyama et al., 1998). The possibility that certain ligands show higher affinity for ERβins compared with ERβ should be considered when novel compounds are tested in rodents.

In addition, for ERβ, alternative translational initiation gives rise to proteins containing 530 and 548 amino acids, respectively, in rodents (Leygue et al., 1998). Only the 530 form has been observed in humans (Xu et al., 2003).

Functional Roles of ERs in Physiology and Disease

Estrogens acting through ERs exert effects on multiple organs. Epidemiological and retrospective studies have provided important evidence for the diverse roles of estrogens in human physiology and disease. There are gender differences in occurrence of several diseases. A role of estrogens in these syndromes is evident from the effects of menopause, when estrogen levels decrease, and estrogen replacement therapy. Furthermore, iden-

tification of phenotypes of ERα, ERβ, and ERα/ERβ knockout (αERKO, βERKO, αβERKO) mice is in many cases consistent with observations in humans and has provided an added molecular understanding of the function of ERs. Some of the effects of estrogens are described below, with a particular focus on clinical conditions.

Breast

ERβ is found in both ductal and lobular epithelial and stromal cells of the rodent (Gotteland et al., 1995). ERα, on the other hand, is only found in the ductal and lobular epithelial cells and not in stroma (Gotteland et al., 1995). It is generally believed that breast tumors depend, at least initially, on the stimulatory effects of estrogens; however, many breast tumors eventually progress to an estrogen-independent growth phenotype. Tamoxifen and similar antiestrogens are currently the first-line therapy for treatment of hormone-dependent breast cancer (Jensen and Jordan, 2003).

Various ER transcripts have been found in breast carcinomas (Gotteland et al., 1995). Protein products corresponding to variant ERs have been described previously (Poola and Speirs, 2001). Normal and cancer tissues display a variety of distinct profiles regarding ERα, ERβ, and splice variants at both mRNA and protein levels (Poola and Speirs, 2001). This heterogeneity in ER isoform profiles is suggested to result in variations in estrogen signaling and might affect breast cancer risk, hormone responsiveness, and survival. Some data suggest that the ERβ transcript is down-regulated in breast tumorigenesis, and other studies show regulation of ERβ expression by promoter methylation (Iwao et al., 2000; Zhao et al., 2003). Since promoter methylation is frequently observed in cancer (Garinis et al., 2002), these data suggest that ERβ is a possible tumor suppressor gene. In vitro studies indicated that ERβ is an important modulator of proliferation and invasion of breast cancer cells, thus supporting the hypothesis that the loss of ERβ expression could be one of the events leading to breast cancer development (Lazennec et al., 2001). However, this hypothesis needs to be confirmed, because it has been shown that ERβ is expressed in the majority of breast tumors, with immunohistochemical staining in approximately 2/3 of breast tumors, similar to the percentage of tumors that express ERα. Currently, only the ERα form is clinically measured for clinical decision-making and treatment.

Prostate

Prostate cancer is the most frequently diagnosed malignancy and the second most common cause of death among men in the United States. The growth and development of the prostate are under endocrine control, in which both androgens and estrogens play important roles. Both ER subtypes are found in the ventral prostate but are located in different cell types (Weihua et al., 2003). ERα is found in the stromal cells, and ERβ is found in the epithelial cells. The estrogenic effects in the prostate may therefore be exerted by both ERs but in different cells. In prostates from βERKO mice, most epithelial cells express the proliferation antigen Ki-67 and the antiapoptopic protein BclII (Imamov et al., 2004). Foci of epithelial hyperplasia appear in mice at 5 months of age, and the frequency of their appearance increases with age (Imamov et al., 2004). In other cases, prostatic hyperplasia is induced by exogenous hormonal treatment such as diethylstilbestrol, but this induced hyperplasia is absent in αERKO, although it is seen in βERKO mice (Couse and Korach, 2004; Taylor et al., 2006).

The Cardiovascular System

Epidemiological and retrospective studies suggest that estrogens exert a cardio-protective role. Women present a higher risk for CVD after the onset of menopause when levels of endogenous estrogen fall (Mendelsohn and Karas, 1999). Accordingly, in some studies, reduced cardiovascular risks have been observed in subjects on hormonal replacement therapy. Estrogens, acting via estrogen receptors in the cardiovascular system, are thought to be important in the prevention of CVD in women. Estrogens have favorable effects on lipid profile, tone of vascular smooth muscle cells, and fibrinogen levels (Khan and Malhotra, 2003). When prescribed alone, estrogen increases the risk of endometrial cancer and is therefore taken in combination with progestins. The Women's Health Initiative reported that estrogen in combination with progestin does not confer cardiac protection and may even increase the risk of CVD among healthy postmenopausal women, especially during the first year of treatment, and both arms of this first prospective study of estrogen and estrogen-progestin for the prevention of cardiovascular disease were terminated early due to an unacceptable risk profile (Hays et al., 2003). However, the timing hypothesis suggests an effect of inclusion criteria with respect to age and that there was a 30% benefit when treatment hormonal replacement therapy was given in perimenopause (Harman et al., 2005). Results from estrogen receptor knockout mice suggest ERα is important in the pathophysiology of the vessel wall (Pare et al., 2002). βERKO mice display a phenotype with abnormalities in ion channel function and an age-related sustained systolic and diastolic hypertension (Zhu et al., 2002).

Bone

ERs are expressed in most cell types in bone (Sims et al., 2002). Estrogen and its receptors are known to be important in the regulation of bone metabolism. Estrogen deficiency beginning at menopause is a major pathogenic factor in the development of osteoporosis in postmenopausal women.

A male patient with a nonfunctional ERα gene showed abnormal postpubertal bone elongation (Smith et al., 1994). Mice lacking the ERα gene show minor skeletal abnormalities with reduced longitudinal bone growth and small reductions in bone mineral density (Sims et al., 2002). Studies of female βERKO indicate that ERβ is responsible for the repression of the growth-promoting effect of estrogen on bone mediated via ERα (Sims et al., 2002). Raloxifene, an estrogen agonist in bone, has been approved for the prevention of postmenopausal osteoporosis.

Estrogen Receptors and Diseases of the Central Nervous System

ERα and ERβ are expressed in the central nervous system (Weihua et al., 2003), and the distribution pattern suggests different functions for the two receptors. In rodents, ERα is mainly distributed in the areas of the central nervous system implicated in the control of reproductive functions such as hypothalamus and preoptic areas. ERβ is more widely distributed, being expressed in areas such as cortex and hippocampus.

Estrogens and their receptors have been implicated in various disorders of the brain, and studies carried out in experimental animals have shown that estrogens exert protective effects against toxic stimuli, ischemic insults, and in disease models. Clinical studies have thus far provided controversial results. Deprivation of estrogen as a result of menopause is associated with an increased risk of Alzheimer's and Parkinson's disease (Bhavnani, 2003). However, the extent to which hormone replacement therapy has positive or negative effects on cognition and in neurodegenerataion (Brinton, 2004) is still quite controversial. Recent studies show a protective effect of estrogens on multiple sclerosis (Soldan et al., 2003; Alonso et al., 2005), most likely due to the immunomodulatory and anti-inflammatory effects of estrogens reported by several laboratories (Cuzzocrea et al., 2000; Liu et al., 2003; Chadwick et al., 2005).

Metabolic Diseases

ERα is expressed in adipocytes. A correlation between estrogen and adipose tissue mass has been seen in both humans and rodents (Lovejoy, 2003). Estrogen reduces food intake and is also known to decrease adipose tissue mass by increasing lipolysis, but the molecular mechanisms for this phenomenon still remain unclear. A similar positive effect of estrogens in protection against insulin resistance and type 2 diabetes has also been described previously (Bailey and Ahmed-Sorour, 1980).

Structural Features of the Ligand-Binding Domain

Several structures have been determined for ERα and ERβ complexed with various ligands and cofactor peptides (Brzozowski et al., 1997). The ER LBD is composed of 12 α helices that form the characteristic three-layer antiparallel α-helical sandwich with a small four-stranded β sheet. Agonists bind to an internal cavity of the receptor that stabilizes the overall conformation of the LBD and induces a conformation of helix 12 that promotes coactivator binding. Coactivator proteins contain one or more of the LXXLL motifs that interact with nuclear receptor AF-2. In the crystal structures, the LXXLL motif adopts an α-helical structure and binds to a cleft on the surface of the LBD formed by helix 3, 5, and 12. Some selectivity in terms of affinity of different LXXLL motifs for ERα or ERβ has been reported.

Although ERα and ERβ share only 56% overall amino acid identity in the LBD, the residues that line the ligand-binding pocket are more conserved with only two amino acid differences. The differences are ERα Met 421 versus ERβ Ile 373 and ERα Leu 384 versus ERβ Met 336. However, amino acid differences not directly part of the ligand-binding pocket as well as the overall smaller size of the ERβ pocket may also affect ligand binding. Importantly, natural and pharmacological ligands exhibit ER subtype selectivity. This selectivity forms the basis for the development of subtype-selective ligands. However, receptor selectivity might not be restricted to the ligand-binding pocket but could also be based on receptor-selective interaction with cofactors. Cofactors include enzymes that affect, for example, nucleosome remodeling by histone acetyl transferases, methylases, and deacetylases that might be particularly well suited targets for pharmacological intervention. Several histone deacetylase inhibitors are being clinically tested for various types of cancer. By contrast, small molecular targeting of protein-protein interactions remains a difficult challenge.

Ligands

Estrogen receptor modulators, agonists, and antagonists have a widespread use in clinical practice today. The total world market for this class of drugs is worth billions of dollars. The introduction of estrogen receptor antagonists for the treatment of hormone responsive breast cancer represents a milestone in the treatment of this life-threatening disease. On the other hand, estrogen receptor agonists are used to alleviate the symptoms associated with postmenopausal syndrome; however, the risk-benefit profile of estrogen substitution therapy is presently under active discussion. Other estrogens to be taken into consideration due to their presence in the environment are pesticides, alkyl phenols, phthalates, and phytoestrogens. Although their affinity for ERs is mostly 100- to 10,000-fold lower than that of estradiol (Kuiper et al., 1998), it is not without question that they could affect human health.

With the recognition of the tissue-selective pharmacology of estrogens, and after the discovery of a second estrogen receptor, we can no longer simply use the word

"estrogenic." If we defined estrogenic in the old way, i.e., stimulation of uterine growth and induction of progesterone receptor in the uterus, ERβ ligands would not qualify as estrogens. If, on the other hand, we defined estrogenic as the ability to directly inhibit prostatic growth, ERα ligands would not qualify as estrogenic. Thus, appropriate terminology to describe the activity of an estrogen ligand needs to make reference to the receptor target (e.g., ERα agonists, ERβ agonists, ERα antagonists, and ERβ antagonists) and to the specific response being considered (i.e., the target tissue, the physiological change, or the gene expression being affected). The term "selective estrogen receptor modulator" (SERM) was developed to provide a more generic description of how the activity of an ER ligand derives from its modulation of ER conformation.

Receptor Subtype-Based Selectivity

Tissue expression and studies on ERα and/or ERβ knockout animals have indicated that the two receptors have distinct biological activities. However, to fully assess the therapeutic prospects of individual subtypes, the effects of selective ligands have to be explored in appropriate animal models.

Shortly after the identification of ERβ, it could be demonstrated that certain compounds show receptor selectivity with regard to ligand binding and/or efficacy. For example, the phytoestrogen genistein is approximately 30-fold ERβ-selective. Propylpyrazole triol has been reported to be 410-fold selective in binding to ERα over ERβ (Stauffer et al., 2000). This compound acts as a potent ERα agonist at concentrations where it has no effect on ERβ. Diarylpropionitrile is an ERβ-selective agonist (Meyers et al., 2001). (*R*,*R*)-THC is an agonist on ERα but an antagonist on ERβ (Meyers et al., 1999; Shiau et al., 2002). Finally, raloxifene is a 15-fold more potent antagonist for ERα than for ERβ (Barkhem et al., 1998).

SERMs

Tamoxifen, introduced for the treatment of breast cancer, represented the first-generation SERM that displays an antagonistic effect on the breast while functioning as an agonist in uterus and skeletal tissue, preserving bone mineral density (Jordan et al., 1987) and with similar beneficial effects on serum lipid and cholesterol profile as 17β-estradiol. Raloxifene, then known as keoxifene, was also shown to preserve bone density in rats (Jordan et al., 1987). One mechanism that contributes to tissue selectivity of tamoxifen is the difference in expression of cofactors in different tissues (Shang and Brown, 2002). Steroid receptor coactivator 1 is not coexpressed with ERα in the mammary epithelium, but the two proteins are coexpressed in the endometrium.

The second-generation SERMs, with improved tissue selectivity, included raloxifene approved for the prevention of postmenopausal osteoporosis. However, there is a need for further development in this field since all desired effects of hormone replacement therapy, including alleviation of menopausal symptoms such as hot flashes, vaginal dryness, and emotional symptoms, are not always efficiently corrected by current drugs.

Biochemical and structural studies have aided our understanding of the SERM nature of tamoxifen and raloxifene. As described above, agonists induce a conformation of helix 12 that promotes coactivator binding. In contrast, in the SERM tamoxifen and raloxifene complexes, the bulky side chains of the compounds sterically prevent helix 12 from taking the agonist position. Instead, helix 12 adopts the position induced by antagonists, overlapping the coactivator binding site and specifically preventing coactivator binding. As SERMs block AF-2, they act as antagonists in cells that depend mainly on AF-2 for activity but could display agonistic properties in cells where AF-1 is active. Cofactor recruitment studies have shown a correlation between agonist activity on a given promoter and the recruitment of coactivators. The lack of activity was instead associated with the recruitment of corepressors. When interacting with the more complete ER antagonist ICI182,780, helix 12 is disordered, and the end of the side chain of the ligand enters the coactivator-binding groove.

Current Evaluation of SERMs as Medicines

Tamoxifen is a pioneering SERM that has been tested exclusively for the treatment of breast cancer and has been successfully evaluated in the United States for the reduction of risk in pre- and postmenopausal women at elevated risk for the disease. Raloxifene, originally described as a nonsteroidal antiestrogen with reduced uterotrophic activity in animals compared with tamoxifen, has been successfully evaluated for the treatment and prevention of osteoporosis and noted to reduce the incidence of breast cancer. Unlike tamoxifen, raloxifene has not been found to increase the risk of endometrial cancer. Raloxifene is currently being evaluated against tamoxifen to determine whether it can reduce the risk of breast cancer in high-risk postmenopausal women. The results of the study of tamoxifen and raloxifene (STAR) were released in June 2006. Compounds classified as SERMs are known to reduce the levels of circulating low-density lipoprotein cholesterol. Raloxifene is currently being evaluated against placebo in women at high risk of coronary heart disease in a study entitled *Raloxifene Used for the Heart*.

The drug toremifene is a SERM with characteristics like tamoxifen, but unlike tamoxifen, the compound does not cause liver tumors in rats. A recent clinical evaluation of toremifene demonstrated equivalence as an adjuvant therapy for breast cancer, but there were twice as many endometrial cancers in the toremifene than in the tamoxifen group (Pagani et al., 2004). Four other

SERMs deserve mention, as they are compounds that have recently been evaluated in the clinic or are currently being evaluated. Lasofoxifene (CP-336156) is currently being tested in worldwide phase III trials to determine whether it can reduce the risk of vertebral fractures, breast cancer, and cardiovascular disease in postmenopausal women. Arzoxifene (LY353381) can broadly be described as a long-acting version of raloxifene, but in recent phase II clinical trials in women with advanced breast cancer and endometrial cancer, arzoxifene proved to be only marginally effective. The results of the STAR trial will clearly be important in evaluating whether arzoxifene should be tested as a potential chemopreventive for breast cancer. Raloxifene has very poor pharmacokinetics and is rapidly excreted from the body. This was the reason it was such a poor breast cancer drug when it was tested in the 1980s. If raloxifene is, in fact, not a significant improvement over tamoxifen in reducing the risk of breast cancer in the STAR trial, then perhaps an agent with better pharmacokinetics such as arzoxifene should be tested. Bazedoxifene has a structure related to raloxifene, and it is currently being evaluated in international phase III clinical trials with the overall goal of the treatment of postmenopausal osteoporosis and a reduction in the risk for breast cancer. Acolbifene (EM652) has a structure similar to raloxifene, but it is administered as the prodrug EM-800. Recent tests demonstrate modest activity in tamoxifen-resistant breast cancer, but since the compound preserves bone density in laboratory animals, there is further potential for testing for the prevention of osteoporosis.

The main features of ERα and ERβ are summarized in Tables 1 and 2, respectively.

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TABLE 1
ERα

Receptor nomenclature	NR3A1
Receptor code	4.10.1:EST:3:A1
Other names	ER1, Esr1
Molecular information	Hs: 595aa, P03372, chr. 6q25[1] Rn: 600aa, P06211, chr. 1q12[2] Mm: 599aa, P19785, chr. 10 A1[3]
DNA binding	
Structure	Homodimer
HRE core sequence	GGTCAnnnTGACC (ERE, half-site, palindrome)
Partners	HSP90 (physical, functional): cellular localization, DNA binding[4]; AP1 (physical, functional): DNA binding, transcriptional activition[5]; SP1 (physical, functional): DNA binding, transcriptional activation[6]
Agonists	Diethylstilbestrol (0.04 nM), 4-hydroxytamoxifen* (0.14 nM), 17β-estradiol* (0.15 nM), propylpyrazole triol (0.23 nM), raloxifen (0.31 nM), estriol (2.2 nM), estrone (3.2 nM), (*R*,*R*)-THC (4.2 nM), tamoxifen* (15 nM) [K_i][7–9]
Antagonists	Hexestrol (0.05 nM), 4-OH-tamoxifen* (0.14 nM), raloxifen (0.31 nM), ICI182780 (0.24 nM), tamoxifen* (15 nM) [K_i][7]
Coactivators	NCOA1, NCOA2, NCOA3, CREBBP, PPARBP, P68, SRA[10]
Corepressors	NCOR1, NRIP1[10]
Biologically important isoforms	ERα46 {Hs}: translation is started at an internal ATG and produces a protein that lacks most of AF1[11]; A908 {Hs}: mutation appears in premalignant breast lesions and malignant breast cancers[12]
Tissue distribution	Endometrium, liver, white adipose tissue, breast, bone, central and peripheral nervous system, ovary, cardiovascular system, brain, testis, prostate, epididymis {Hs, Mm, Rn} [Northern blot, Q-PCR, in situ hybridization, Western blot, immunohistology]
Functional assays	Uterotrophic assay {Mm, Rn}[13]; HGP axis (gonadotropin suppression) {Hs, Mm, Rn}[14]; vaginal cornification {Mm, Rn}[15]
Main target genes	Activated: pS2 {Hs}[16], progesterone receptor {Hs}[17], cathepsin D {Hs}[18]
Mutant phenotype	Obesity, insulin resistance, diabetes, infertility (male and female), uterine atrophy, female loss of negative gonadotropin feedback, loss of female sexual and maternal behavior, genu valgum, elevated gonadotropins, autoimmune glomerularnephritis, osteopenia, cardiovascular vasodilation resistance {Mm} [knockout][19]
Human disease	Breast cancer[20]; endometrial cancer[21]; obesity[22]; insulin resistance and diabetes[22]

aa, amino acids; chr., chromosome; HRE, hormone response element; Q-PCR, quantitative polymerase chain reaction; CREBBP, cAMP response element binding protein binding protein; PPARBP, peroxisome proliferator-activated receptor binding protein; SRA, steroid receptor RNA activator; HGP, hypothalamus-gonadotropin-pituitary axis.

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TABLE 2
ERβ

Receptor nomenclature	NR3A2
Receptor code	4.10.1:EST:3:A2
Other names	ER2, Esr2
Molecular information	Hs: 530aa, Q92731, chr. 14q23[1] Rn: 530aa, Q62986, chr. 6q24[2] Mm: 530aa, O08537, chr. 12 D1–D3[3]
DNA binding	
Structure	Homodimer
HRE core sequence	GGTCAnnnTGACC (ERE, half-site, palindrome)
Partners	HSP90 (physical, functional): cellular localization, DNA binding[4]; AP1 (physical, functional): DNA binding, transcriptional activition[5]; SP1 (physical, functional): DNA binding, transcriptional activation[6]
Agonists	Diethylstilbestrol (0.17 nM), 17β-estradiol* (0.46 nM), diarylpropionitrile (2.5 nM), estriol (4.0 nM), genistein (6.2 nM), estrone* (11.5 nM), tamoxifen* (68 nM) [K_i][7–8]
Antagonists	4-OH-Tamoxifen* (0.023 nM), (*R*,*R*)-THC (1.45 nM), ICI18780 (1.83 nM), raloxifen (13.3 nM), HPTE antagonist (23 nM), tamoxifen* (68 nM) [K_i][7,9,10]
Coactivators	NCOA1, NCOA2, NCOA3, CREBBP, PPARBP, P68, SRA[11]
Corepressors	NCOR1, NRIP1[11]
Biologically important isoforms	ERβCX {Hs}: splicing variant for last exon—changes the amino acid sequence, resulting in reduced ligand binding[12]; ERβins {Mm, Rn}: splice variant, 18 amino acids inserted between exons 5 and 6[13]
Tissue distribution	Prostate, ovary, lungs, mammary gland, bone, uterus, epididymis, kidney, bladder, intestine, central and peripheral nervous system {Hs, Mm, Rn} [Northern blot, Q-PCR, in situ hybridization, Western blot, immunohistology]
Functional assays	Defective ovulatory stimulation {Mm}[14]
Main target genes	Activated: pS2 {Hs}[15], progesterone receptor {Hs}[16], cathepsin D {Hs}[17]
Mutant phenotype	Reduced fertility (female), prostate hyperplasia, disturbed neuronal migration in embryonic brain, myeloproliferative disease, hypertension {Mm} [knockout][18]

aa, amino acids; chr., chromosome; HRE, hormone response element; HPTE, 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane; Q-PCR, quantitative polymerase chain reaction; CREBBP, cAMP response element binding protein binding protein; PPARBP, peroxisome proliferator-activated receptor binding protein; SRA, steroid receptor RNA activator.

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