

## Review

# Estrogen receptor transcription and transactivation Basic aspects of estrogen action

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## Abstract

Estrogen signaling has turned out to be much more complex and exciting than previously thought; the paradigm shift in our understanding of estrogen action came in 1996, when the presence of a new estrogen receptor (ER), ER $\beta$ , was reported. An intricate interplay between the classical ER $\alpha$  and the novel ER $\beta$  is of paramount importance for the final biological effect of estrogen in different target cells.

**Keywords:** breast, central nervous system, estrogen receptor  $\beta$ , estrogen receptor knockout mice, heterodimerization, prostate, uterus

## Introduction

Jensen and Jacobsen were the first to describe that the biological effects of estrogen are mediated by a receptor protein [1]. The cloning of the ER, today renamed ER $\alpha$ , was reported in 1986 [2,3]. For a long time, it was believed that only one ER existed; however, in 1995 a second ER, ER $\beta$ , was cloned from a rat prostate cDNA library by Gustafsson and colleagues [4\*]. This finding has led to a paradigm shift in our understanding of estrogen action, as will be evident from the different reviews in this issue of *Breast Cancer Research*.

## ER $\beta$ and ER $\alpha$ isoforms

Since the discovery of ER $\beta$  in rat prostate, several groups have reported the cloning of ER $\beta$  from other species [5–7] or different sized ER $\beta$  isoforms, some with extended N-termini and others with truncations and/or insertions in the C-terminal ligand binding domain (LBD). The original

ER $\beta$  clone encodes a protein of 485 amino acids, designated ER $\beta$ -485. ER $\beta$ -503 has an 18 amino acid residue in frame insertion into the LBD, and has a considerably lower affinity for E2 than ER $\beta$ -485. Both ER $\beta$ -503 and ER $\beta$ -485 bind to a consensus estrogen response element (ERE) and heterodimerize with each other and with ER $\beta$  [8,9]. The coactivator SRC-1 interacts with both ER $\alpha$  and ER $\beta$ -485 in an estrogen-dependent manner but not with ER $\beta$ -503 [9]. An additional ER $\beta$  isoform, ER $\beta$ cx [10], is identical to ER $\beta$ -503 except that the last 61 C-terminal amino acids (exon 8) are replaced by 26 unique amino acid residues. The ER $\beta$ cx isoform shows no ligand binding activity and has no capacity to activate transcription of an estrogen-sensitive reporter gene [10]. Furthermore, ER $\beta$ cx shows preferential heterodimerization with ER $\alpha$  rather than with ER $\beta$ , inhibiting ER $\alpha$  DNA binding and having a dominant negative effect on ligand-dependent ER $\beta$  reporter gene transactivation [10].

Various alternatively spliced forms of ER $\alpha$  have also been reported [11–16]. Whether all isoforms or differentially spliced versions of ER $\alpha$  and ER $\beta$ , respectively, are expressed as proteins or have any major biological role warrants further investigation.

ER $\alpha$  and ER $\beta$  are similar in their architecture to the other members of the steroid/thyroid hormone nuclear receptor superfamily [17–22] in that they are composed of independent but interacting functional domains. Ligand-induced gene modulation by hormone receptors is due to ligand-induced conformational changes in the receptor. These conformational changes lead to receptor dimerization, receptor–DNA interaction, recruitment of and interaction with co-activators and other transcription factors, and the formation of a preinitiation complex [23–26].

In ER $\alpha$ , the N-terminal A/B domain encodes activation function 1 (AF1) [27–30]. Synthetic antiestrogens such as tamoxifen, raloxifene and ICI 164,384 induce a partial agonism on an ERE-based reporter gene in the presence of ER $\alpha$  but pure estrogen antagonism with ER $\beta$  [7,31\*,32]. In ER $\alpha$ , different parts of AF1 are required to mediate the agonism of E2 and the partial agonism of tamoxifen [30], a particular function of ER $\alpha$  AF1 that is missing in ER $\beta$  [32]. Differences in the amino-terminal regions of ER $\alpha$  and ER $\beta$  thus constitute a possible explanation for the difference between ER $\alpha$  and ER $\beta$  in their response to various estrogens including antagonists such as tamoxifen and raloxifene.

The C or DNA binding domains of ER $\alpha$  and ER $\beta$  are highly homologous [6] with identical P-box sequences and, therefore, ER $\alpha$  and ER $\beta$  are likely to bind to different EREs with similar specificity and affinity.

Activation function 2 (AF2) in the LBD constitutes the ligand-dependent transcription activation function of nuclear receptors [26,33–37]. In the crystal structure of ER $\alpha$  LBD, complexed with E2 [38\*\*], the agonist-induced positioning of H12 over the ligand-binding pocket has been shown to form the basis for the AF2 coactivator recruitment and interaction surface, together with amino acid residues in H3, H4, and H5. In contrast, in the ER $\alpha$  and ER $\beta$  LBD–raloxifene complexes, respectively [38\*\*,39], H12 was displaced from its agonist position over the ligand-binding cavity and instead occupied the hydrophobic groove formed by H3, H4, and H5, foiling the coactivator interaction surface. Although E2 and raloxifene bind to the same cavity in the receptor, these ligands induce a different conformation of H12 in the LBD, discriminating an agonistic effect by E2 from estrogen antagonism by raloxifene. Surprisingly, H12 in the ER $\beta$  genistein structure did not adopt an agonist conformation [39] but a position more similar to an antagonist conformation, a finding in agreement with the partial (60–70% of E2) agonism of genistein acting via ER $\beta$  on an ERE-driven reporter gene in cells [31\*]. It is evident that

different ligands induce different receptor conformations [24,40], and that different conformations of the receptor affect the agonist efficacy and potency of ligands.

An interesting difference between ER $\alpha$  and ER $\beta$  is also seen on an AP1 site. In the presence of ER $\alpha$ , typical agonists such as E2 and diethylstilbestrol as well as the antiestrogen tamoxifen function as equally efficacious agonists in the AP1 pathway, raloxifene being only a partial activator. In contrast, in the presence of ER $\beta$ , the antiestrogens tamoxifen and raloxifene behave as fully competent agonists in the AP1 pathway, while estradiol acts as an antagonist inhibiting the activity of both tamoxifen and raloxifene [41\*\*].

### Tissue distribution of ER $\beta$ and ER $\alpha$

ER $\beta$  is widely distributed in the organism. ER $\beta$  was originally cloned from rat prostate, which is one of the most ER $\beta$  dense in the body. The ovaries in the female rodent show a corresponding abundance of ER $\beta$ , mainly in the granulosa cells. The tissues that appear to be richest in ER $\beta$  are the central nervous system, the cardiovascular system, the lung, the kidney, the urogenital tract, the mammary gland, the colon, the immune system and the reproductive organs. The significance of ER $\beta$  and ER $\alpha$  in some tissues will now be discussed.

### Breast tissue

The importance of estrogens in the development of female breast tissue is well documented. Female aromatase deficient patients, unable to convert C<sub>19</sub> steroids (eg testosterone) to estrogens, showed no sign of breast development at the onset of puberty [42–44]. Administration of estrogen to the two described female patients, however, led to normal prepubertal and postpubertal breast development. ER $\alpha$  knockout female mice have lost their capacity to develop mammary gland tissue beyond the embryonic and fetal stages despite elevated levels of circulating estrogens (17 $\beta$ -estradiol).

More than 70% of primary breast cancers in women are 'ER' (actually ER $\alpha$ ) positive and show estrogen-dependent growth [45], and undergo regression when deprived of supporting hormones. Patients whose breast tumors lack significant amounts of ER rarely respond to endocrine ablation or treatment with antiestrogens, whereas most patients with ER-containing cancers benefit from such treatment [46,47]. Immunochemical determination of ER in tumor biopsies has become a routine clinical procedure on which the choice of therapy is based. However, the currently available immunochemical procedures for ER measurements are based on ER $\alpha$ -specific antibodies that do not detect ER $\beta$  protein (unpublished observations).

ER $\beta$  mRNA and protein have been detected in human breast cancer biopsies and in human breast cancer cell

lines [6,48–50]. With the use of receptor specific antibodies, both ER $\alpha$  and ER $\beta$  were expressed in the normal rat mammary gland, but the presence and cellular distribution of the two receptors was distinct [51\*]. Furthermore, while the level and number of cells expressing ER $\beta$  were more or less constant during prepubertal and pubertal stages, and throughout pregnancy, lactation and postlactation, the level and percentage of ER $\alpha$ -containing cells varied dramatically. The possible role of ER $\beta$  in normal breast tissue development and physiology or in breast cancer development and/or therapy is, however, as yet unknown [52,53\*].

### Urogenital tract

Estrogens are claimed to be effective in the treatment of urge incontinence in postmenopausal women (see [54,55] and references cited therein). It has recently been shown that ER $\beta$  is highly expressed in the inner epithelial cell layer of the rat bladder and urethra [56,57], which may explain the beneficial effect of estrogens in urinary incontinence and suggest that patients with urinary incontinence might benefit from ER $\beta$ -selective agonist therapy.

Estrogens have been linked with prostate pathologies. It has been shown in different species that estrogens synergize with androgens in inducing glandular hyperplasia and dysplasia, and adenocarcinoma in the prostate [58\*]. Immunohistochemical studies have revealed that ER $\beta$  is the predominant ER in the prostate, located in the epithelial cells along the ductal network of the prostate. ER $\alpha$  has been detected only in the stromal compartment of the prostate [57,58\*] (Weihua *et al*, manuscript submitted). ER $\beta$ -/- mice display signs of prostatic hyperplasia with aging [59]. This suggests that ER $\beta$  may protect against abnormal prostate growth and that ER $\beta$ -selective ligands would be of clinical relevance in the prevention and treatment of neoplasia of the prostate.

### Bone: development and homeostasis

There is compelling evidence that estrogens protect postmenopausal women from bone loss and the development of osteoporosis, maintaining a balance between bone resorption and bone formation [54,55,60–63]. As in other tissues, estrogens probably have both direct and indirect effects in maintaining a balanced bone metabolism. The likelihood of important direct effects of estrogens on bone is based on the presence of ER $\alpha$  in the bone-forming osteoblasts [64–66] and in the bone-resorbing osteoclasts [67]. ER $\beta$  mRNA has been found in primary rat osteoblasts and in rat osteosarcoma cells [68]. It has been described in immortalized human fetal osteoblasts that ER $\alpha$  and ER $\beta$  are differentially expressed during osteoblast differentiation *in vitro* [69].

### The cardiovascular system

The risk of women developing cardiovascular disease increases dramatically after the menopause, suspected to

be a consequence of the cessation of estrogen production by the ovaries. Estrogen replacement therapy has a cardiovascular protective effect in postmenopausal women, significantly decreasing the risk of developing atherosclerosis and cardiovascular disease [54,55,70–74].

The estrogen receptors ER $\alpha$  and ER $\beta$  are expressed in vascular endothelial cells [74–76], smooth muscle cells [77–79], and in myocardial cells [56,80]. Various direct effects of estrogen on vascular tissue have been reported [73,74,80–82]: nongenomic vasodilatation as an effect of estrogen on ion channel function [83] and nitric oxide synthesis [84–87]; long-term effects by modulation of, for example, prostaglandin synthase, nitric oxide synthase and endothelin gene expression [88–93]; regulation of AT1 receptor density on vascular smooth muscle cells [94]; and inhibition of injury-induced vascular intimal thickening [95–97]. Furthermore, reduced heart contractility in ovariectomized female rats was normalized following estrogen replacement [98], an effect explained in part by estrogen mediated changes in expression of contractile proteins [80,99]. The precise functions of ER $\alpha$  and ER $\beta$  in protection of the vessel wall from injury-induced hyperproliferation are still under active investigation. Estrogen can inhibit hyperproliferation of the vascular smooth muscle cells after injury in both ER $\alpha$  knockout and BERKO (ER $\beta$ -/-) mice [100\*–102\*], possibly indicating that the effects of estrogen on the smooth muscle cells are not receptor mediated, but possibly also indicating that the vessel wall is one location where ER $\alpha$  and ER $\beta$  have overlapping functions. The answer to the question will be found when ER $\alpha$ /ER $\beta$  double knockout mice are examined.

### Central nervous system and the hypothalamus–pituitary axis

Estrogens are reported to influence a variety of functions in the central nervous system such as learning, memory, awareness, fine motor skills, temperature regulation, mood, and reproductive functions [103]. Estrogens are also linked to symptoms of depression and treatment of depressive illness.

The expression patterns of ER $\alpha$  and ER $\beta$ , respectively, based on mRNA, autoradiographic or immunohistochemical studies of rat and mouse brain, indicate that there is selective expression of one of the two ER subtypes in certain areas of the brain, but that there are also areas where they seem to be colocalized. ER $\alpha$  is more abundant in the hypothalamus (preoptic, arcuate, periventricular, and ventromedial nucleus) and in selected nuclei in the amygdala (hippocampal area, medial and cortical nucleus) [104–107]. A high level of ER $\beta$  mRNA has been found in the medial preoptic, paraventricular and supraoptic nucleus of the rat hypothalamus and in the medial amygdala nucleus. Moderate to high ER $\beta$  mRNA is expressed in olfactory bulbs, the bed nucleus of the stria terminalis, the hippocampus, the cerebral cortex, the cerebellum, the midbrain raphe and the basal forebrain [103,105–111].

The hypothalamus–pituitary axis regulates overall endocrine homeostasis in the body. Estrogen, through effects on the hypothalamus–pituitary axis, modulates the expression and secretion of hormones such as luteinizing hormone, follicle stimulating hormone, growth hormone, and prolactin, from the anterior pituitary gland [112]. Both ER $\alpha$  and ER $\beta$  are expressed in the pituitary gland but ER $\alpha$  predominates [112,113], particularly in the gonadotrophs and lactotrophs. Both ER subtypes are also expressed in the preoptic area of the hypothalamus, which is involved in regulating the expression of pituitary hormones, but ER $\beta$  is predominant [105].

### Concluding remarks

Our understanding of estrogen action has undergone a radical change following the discovery of ER $\beta$ . Although not addressed in this particular review, evidence is accumulating that ER $\alpha$  and ER $\beta$  may indeed regulate, at least partially, separate and distinct gene networks. We are thus now beginning to have tools to grasp many of the seemingly confusing and contradictory aspects of estrogen action, particularly regarding tissue specific and cell specific effects of estrogen. Varying ratios between ER $\alpha$  and ER $\beta$  in different contexts seem to quite probably be of paramount importance for the finally obtained hormonal effects. This paradigm shift in our concepts of estrogen action, needless to say, will lead to many exciting new opportunities for pharmaceutical development in the field of women's health.

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