Nuclear receptors in disease: the oestrogen receptors

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Abstract

For several decades, it has been known that oestrogens are essential for human health. The discovery that there are two oestrogen receptors (ERs), ERα and ERβ, has facilitated our understanding of how the hormone exerts its physiological effects. The ERs belong to the family of ligand-activated nuclear receptors, which act by modulating the expression of target genes. Studies of ER-knockout (ERKO) mice have been instrumental in defining the relevance of a given receptor subtype in a certain tissue. Phenotypes displayed by ERKO mice suggest diseases in which dysfunctional ERs might be involved in aetiology and pathology. Association between single-nucleotide polymorphisms (SNPs) in ER genes and disease have been demonstrated in several cases. Selective ER modulators (SERMs), which are selective with regard to their effects in a certain cell type, already exist. Since oestrogen has effects in many tissues, the goal with a SERM is to provide beneficial effects in one target tissue while avoiding side effects in others. Refined SERMs will, in the future, provide improved therapeutic strategies for existing and novel indications.

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The oestrogen receptors (ERs)

For several decades, it has been known that the steroid hormone oestrogen is involved in disease. As early as 1896, Dr George Beatson conducted experiments in which removal of the ovaries from post-menopausal women with advanced breast cancer, led to shrinkage of the tumour and improved prognosis (reviewed in [1]). In 1916, ovariectomy of mice with a propensity to develop mammary carcinoma was found to reduce the incidence of tumours. In 1923, it was discovered that oestrogenic hormones are produced by the ovaries. In the late 1950s, the existence of a receptor molecule that could bind 17β-oestradiol was discovered by Jensen and Jacobson (reviewed in [1]); in 1986, the first ER was cloned [2,3]. This receptor was regarded as the only existing ER for 10 years, until members of our laboratory discovered a second ER [4]. The two receptors are today known as ERα and ERβ respectively, and belong to the family of ligand-activated nuclear receptors. These receptors exert their effects by modulating the expression of target genes. ERα and ERβ show a high degree of similarity when compared at the amino-acid level (Figure 1); however, it is clear that the receptors display differences with regard to ligand-binding and transcriptional activation. The receptors 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. ERα is, for example, the major receptor in the liver, while ERβ is the main receptor in the intestines. Both receptors can be found in heart and bone. Also, both receptor subtypes may be present in the same tissue, but in different cell types. For instance, ERα is found in the stromal cells and ERβ in the epithelial cells of the prostate. Mice lacking one or both of the receptor subtypes have been, and still are, instrumental in increasing our understanding of the role of oestrogen and its receptors in physiology and disease [5]. The ER-knockout (ERKO) mice display varying phenotypes in bone, brain, mammary gland, immune system, cardiovascular system and

![Figure 1. Comparison of the human ERs (hER) at the amino acid level](image)

Percentage amino acid identity between the two receptor types is indicated in the model for hERβ. ERα is 595 amino acids long and ERβ WT (ERβ1) is 530 amino acids long. N, N-terminal domain; DBD, DNA-binding domain; HINGE, hinge domain, which bridges the DBD and ligand-binding domain (LBD); C, the very C-terminal domain, which harbours transactivation activity.
prostate, all of which will be discussed further in this review. Both male and female αERKO and αβERKO mice are completely infertile, whereas βERKO males exhibit normal fertility [5]; βERKO females show decreased fertility [6]. The infertility in the ERKO models can be partly accounted for by an inability to ovulate; in fact, both ERs are required for ovulation to occur efficiently.

**ER modulators in physiology and disease**

ER 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 ER antagonists for the treatment of hormone-dependent breast cancer represents a milestone in the treatment of this life-threatening disease. ER agonists are often used to alleviate the symptoms associated with post-menopausal syndrome. However, the risk–benefit profile of this substitution therapy needs to be considered.

The following sections focus on some selected diseases in which oestrogen and its receptors have been implicated.

**ERs and breast cancer**

It is generally believed that breast tumours, at least initially, are dependent on the stimulatory effects of oestrogens, directly or indirectly, and it is thought that oestrogens act by inducing the expression of paracrine growth factors and their receptors. However, many breast tumours eventually progress to an oestrogen-independent growth phenotype. Tamoxifen and similar anti-oestrogens are currently the first-line therapy for treatment of hormone-dependent breast cancer [1].

Various ER transcripts have been found in breast carcinomas [7,8] and protein products corresponding to variant ERs have been described [9]. Splicing of ERα precursor RNA frequently leads to variants, lacking one or more exons, that have been associated with breast cancer progression. The most frequent splice variants are exon 4 or exon 7 deletions [10]. Exon 4 comprises the hinge region and the very N-terminal part of the ligand-binding domain (LBD), and exon 7 contains part of the LBD. Of these two, only the exon 7 deletion has so far been detected at the protein level [9]. Single exon deletions in ERα can be found in a majority of normal breast tissues, while an increased frequency of multiple exon deletions is found in tumours [9]. Normal and cancerous tissues display a variety of distinct profiles regarding ERα wild-type (WT), ERβ WT, and ERα and ERβ splice variants at both mRNA and protein levels. This heterogeneity in ER isoform profiles is suggested to result in variations in oestrogen signalling, and might affect breast cancer risk, hormone-responsiveness and survival [9]. Some data suggest that the ERβ1 (ERβ WT) transcript is down-regulated in breast tumorigenesis [11–13], but this was not shown to be consistent for all tumour grades [14]. A higher level of ERβ2 (ERβ CX), compared with the ERβ1

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transcript, was reported in human breast cancer cell lines, as well as in breast tumours and normal breast tissue [12–15]. ERβ CX is a variant of ERβ WT in which the last exon (LIV; see Figure 2B) is spliced away and replaced with the CX exon. We have shown that methylation of the ERβ promoter is inversely correlated with ERβ expression in breast cancer tumours and cell lines [15]. Since promoter methylation is frequently observed in cancer, these data suggest that ERβ is a possible tumour-suppressor gene.

ERβ is found in ductal, lobular epithelial and stromal cells of the rodent mammary gland (reviewed in [16]). ERα, on the other hand, is only found in the ductal and lobular epithelial cells, and not in the stroma. The αERKO mice display severe retardation of mammary gland development and show rudimentary glands at adulthood [16]. Studies of the βERKO mice indicate that ERβ is not necessary for ductal growth of the gland, but seems to be important for the organization and adhesion of epithelial cells [16].

**ERs and prostate cancer**

Prostate cancer is the most frequently diagnosed malignancy and the second most common cause of death among men in the U.S.A. Prostate cancer is age-dependent and the incidence increases after the age of 40. The growth and development of the prostate is under endocrine control and both androgens and oestrogens play important roles [17]. Androgens are essential for stimulating normal development, growth and secretory activities of the prostate, whereas oestrogens are generally regarded as inhibitors of growth. Combined androgen and oestrogen treatment has been shown to induce prostatic dysplasia and adenocarcinoma [18]. These results demonstrate that malignant changes in the prostate gland may be dependent upon both androgenic and oestrogenic effects. Oestrogens have been shown to induce growth of LNCaP prostate cancer cells, which can be inhibited by anti-oestrogens [19]. However, this cell line contains a mutant androgen receptor (AR), which results in promiscuity in hormone binding and receptor activation, making interpretation of these results difficult. In an androgen-decreased environment, oestrogen, through ERα, causes benign prostate hyperplasia, dysplasia and cancer [20]. Studies have shown that anti-oestrogens and specific ER modulators (SERMs) can delay and suppress prostate carcinogenesis [20]. Both ER subtypes are found in the ventral prostate, but are located in different cell types [16]. ERα is found in the stromal cells and ERβ in the epithelial cells, in which ERα is absent. The oestrogenic effects in the prostate may therefore be exerted by both ERs, but in different cells. Studies of transgenic mice show interesting phenotypes with hyperplastic prostates in both βERKO and aromatase-knockout (ARKO) mice, but not in αERKO mice [16]. In prostates from βERKO mice, most epithelial cells express the proliferation antigen Ki-67, and the tissue contains several hyperplastic foci [16]. In contrast, the
prostate epithelium of their WT littermates show no hyperplasia and with only a few cells expressing Ki-67 [16]. The hyperplasia is thought to be directly caused by lack of ERβ and its anti-proliferative function in the prostate. The hyperplasia in ARKO mice may also be due to increased androgen and prolactin levels, secondary effects caused by the general loss of aromatase, the enzyme that converts androgens into oestrogens [16].

**ERs and cardiovascular disease**

Women present a higher risk for cardiovascular disease (CVD) after the onset of menopause, a phenomenon thought to be due to the loss of endogenous oestrogen. Accordingly, in some studies, reduced cardiovascular risks have been observed in subjects undergoing hormonal replacement therapy (HRT). Oestrogens, acting via ERs in the cardiovascular system [21], are thought to be important in prevention of CVD in women. Oestrogens have favourable effects on lipid profile, tone of vascular smooth muscle cells and fibrinogen levels [22]. When prescribed alone, however, oestrogen increases the risk of endometrial cancer and is therefore taken in combination with progestins, which are anti-proliferative in the uterus. Importantly, however, an oestrogen–progestin arm of the first prospective study of oestrogen and oestrogen–progestin for prevention of CVD was terminated early owing to an unacceptable risk profile. The Women’s Health Initiative (WHI) reported that oestrogen in combination with progestin does not confer cardiac protection and may even increase the risk of CVD among healthy post-menopausal women, especially during the first year of treatment [23]. Furthermore, there was an increased risk of ischaemic stroke in generally healthy post-menopausal women [24]. However, the oestrogen-alone arm of the study is on-going. Results from the study of ERKO mice suggest that ERα is important in the pathophysiology of the vessel wall [25]. The βERKO mice display a phenotype with abnormalities in ion-channel function and an age-related sustained systolic and diastolic hypertension [25].

**ERs and osteoporosis**

Oestrogen and its receptors are known to be important in the regulation of bone metabolism. Oestrogen deficiency beginning at the menopause is a major pathogenic factor in the development of osteoporosis in post-menopausal women. The ERs are expressed in most cell types in bone [26]. A male patient with a non-functional ERα gene showed abnormal post-pubertal bone elongation [27]. Mice lacking the ERα gene show minor skeletal abnormalities with reduced longitudinal bone growth and small reductions in bone mineral density (BMD) [26]. Studies of female βERKO mice, which lack ERβ, indicate that ERβ is responsible for the repression of the growth-promoting effect of oestrogen on bone mediated via ERα [26].
ERs and diseases of the central nervous system (CNS)

ERs have also been implicated in various disorders of the brain. The receptors are expressed in the CNS, where they are thought to play important roles [16]. The distribution pattern suggests different functions for the two receptors. βERKO mice show an interesting phenotype with severe neuronal deficiency in the cortex, revealing an important role for ERβ in neuronal migration [16,28]. αERKO mice show no morphological abnormalities in the brain. Oestrogen has been proposed to act as a neuroprotectant. Deprivation of oestrogen as a result of the menopause is associated with an increased risk of Alzheimer’s disease and Parkinson’s disease [29]. Oestrogen replacement therapy may reduce this risk in both men and women.

ERs and diseases of the immune system

Oestrogen and its receptors also play important roles in the immune system. Ovariectomized mice show splenomegaly [30] and an increased production of haematopoietic cells [31]. Proliferation of pluripotent bone marrow stem cells is negatively regulated by oestrogen [31]. βERKO mice develop pronounced splenomegaly by 1.5 years of age [31], a phenomenon that is much more severe in females than in males. Interestingly, the absence of ERβ results in a myeloproliferative disease resembling human chronic myeloid leukaemia with lymphoid blast crisis. These intriguing results suggest a role for ERβ in regulating the pluripotent haematopoietic progenitor cells and make the βERKO mice a potential model for lymphoid and myeloid leukaemia [31].

ER polymorphisms and mutations in relation to disease

A few studies have been published in which the ERα and ERβ genes have been screened to identify mutations which change the amino acid sequence of the proteins, and are therefore candidates to change receptor function. Various ERα variants, including deletion variants, have been found in breast carcinomas [9]. A point mutation in the ERα gene that generates a stop codon, resulting in a truncated ERα protein, has been reported in one male patient [27]. This patient is suffering from osteoporosis and infertility. In a systematic mutation screening of ERβ in probands of different mass extremes, five different genetic variants were identified [32]. Our laboratory has screened approx. 50 patients diagnosed with various infertility syndromes such as polycystic ovary syndrome, premature ovarian failure and endometriosis, for mutations in the ERβ exons, only to reveal a few new variations, none of which cause a change in the primary structure of the protein (M. Nilsson and M. Zelada-Hedman unpublished work). We have also screened 34 primary breast cancer specimens without detecting any novel variants (M. Nilsson, unpublished work).
Single-nucleotide polymorphisms (SNPs) are base-pair changes that exist naturally in a population. Usually, variants that occur at an allele frequency exceeding 1% are referred to as SNPs. SNPs provide important tools for human genetic studies. A set of polymorphisms are scored in cases and controls. If an association is detected, i.e. a specific nucleotide is more common at one position in the gene in cases compared with controls, this indicates that the polymorphism itself, or some other change in the same or neighbouring genes, is related to the disease. A number of polymorphisms have been reported in the ERs. An overview of commonly scored ERα and ERβ variants is given in Figure 2. Generally, these SNPs do not change the amino acid sequence of the resulting ER proteins and thus are not likely to change receptor function. However, that they change the expression level of the receptor proteins cannot be excluded. A number of case-control studies have investigated a possible association between ERα or ERβ SNPs and disease. Studies of coronary artery disease (CAD) patients failed to show an association with the ERα variants B and C (see Figure 2A) [33]. Conflicting results have been reported for association between the ERα variants E and F (Figure 2A) and BMD [34], probably because of differences in screened populations. Another study reported that ERα variant B (Figure 2A) might be a risk factor for prostate cancer [35]. We have shown an association between ERβ SNPs I and J (Figure 2B) and bulimic patients [36], and others have shown an association between ERβ D and anorexia nervosa [37].

**Figure 2. SNPs at the human ERα (A) and ERβ (B) gene loci**

(A) E1–E8, exons 1–8; A, (TA)n repeats (17 alleles); B, codon 10 C/T (MspI RFLP); C, codon 87 G/C (BstUI RFLP); D, codon 160 C/T; E, intron 1 A/G (XbaI RFLP); F, intron 1 C/T (PvuII RFLP); G, codon 311 G/A; K, codon 335 C/T; L, (CA)n repeats (9 alleles); M, codon 425 C/T; N, codon 594 A/G. (B) A, N-terminal exon; CI, first part (finger) of the DNA-binding domain; CII, second part (finger) of the DNA-binding domain; HIN, hinge exon; LI–LIV, ligand-binding domain exons; CX, CX exon; A, 661 A/G; B, 809 (del21); C, 846 G/A; D, 1082 G/A; E, (CA)n repeat; F, 1421 T/C; G, ERβ LIV – 68 C/T; H, ERβ LIV – 4 A/G; I, 1730 G/A; J, ERβ CX + 56 G/A. The ERβ LIV – 68 and –4 polymorphisms are located 68 and 4 nucleotides 5' of exon LIV respectively, and the ERβ CX + 56 is located 56 nucleotides 3' of exon CX.
Interestingly, ERβ is located on chromosome 14, in a recently identified region that meets the criteria for genome-wide suggestive linkage with bulimia nervosa [38]. However, a German study did not provide evidence for an association between bulimia nervosa and ERβ polymorphisms D and I [32]. Other studies have investigated the possible association between a dinucleotide repeat polymorphism (E in Figure 2B) located in the flanking region of the human ERβ gene and various clinical parameters. A possible association between this polymorphism and hypertension in Japanese women was reported in [39]. The dinucleotide repeat was also suggested to be associated with BMD [40] and androgen levels in women [41], but not with autoimmune thyroid diseases in another study [42]. Association studies of the same ERβ gene polymorphism found a correlation with higher BMD in pre-menopausal, but not post-menopausal Chinese women [43], suggesting that the ERβ gene may have a modulatory role in bone metabolism in young adulthood. Six different polymorphisms in ERβ (B, C, D, E, H and I) were studied in a sporadic breast cancer material; however, no differences were found in the allelic distribution of the six studied polymorphisms between the breast cancer and control groups [44].

**Conclusion**

The ERs are known to have important roles in many different diseases, including osteoporosis, prostate cancer, breast cancer, cardiovascular disease and diseases of the CNS. Recently, other interesting functions of the ERs have been proposed. Studies of mice revealed that absence of ERβ results in a myeloproliferative disease resembling human chronic myeloid leukaemia with lymphoid blast crisis. This might indicate a role for ERβ in regulating the differentiation of pluripotent haematopoietic progenitor cells. Future association studies on large populations will contribute to our understanding of the role of ERα and ERβ for the development of disease in humans. We are expecting an explosion in our understanding of the molecular effects of oestrogen in target tissues in relation to physiology and disease. This will be possible using modern gene expression profiling and proteomics technologies. Furthermore, receptor-selective ligands, which are currently being developed, will provide important tools to understand ER biology and studies in ERα- and/or ERβ-deficient mice will clarify the importance of a specific receptor for a given effect. These studies will aid the development of SERMs with well-characterized and optimal effects in oestrogen-responsive tissues. SERMs are already available; however, existing SERMs are modest in their desired tissue-selective activity and have undesired side effects. Therefore a great need exists for novel SERMs with a more refined therapeutic profile with optimal selectivity and activity in oestrogen-responsive tissues.
Summary

- The ERs have been found to play important roles in many different diseases, including osteoporosis, prostate cancer, breast cancer and CVD. Oestrogen is suggested to have a neuroprotective role. The onset of the menopause most probably leads to an increased risk of neurodegenerative disorders, such as Parkinson’s and Alzheimer’s diseases, in women.

- The ERKO mice display interesting phenotypes, including alterations in bone, breast, CNS, immune system, reproductive organs and prostate.

- ER variants have been identified, only a few of which result in changes in amino acid sequence.

- Association studies have shown correlations between ERα/β polymorphisms and bulimia, anorexia, BMD and hypertension.

- The development of SERMs with well-characterized and optimal effects in oestrogen-responsive tissues will be very important in the treatment of ER-dependent disorders.

References


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