Review

Estrogen dependent signaling in reproductive tissues – A role for estrogen receptors and estrogen related receptors

Douglas A. Gibson, Philippa T.K. Saunders ∗

MRC/UoE Centre for Reproductive Health, The University of Edinburgh, The Queen’s Medical Research Institute, Edinburgh EH16 4TJ, UK

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A B S T R A C T

Estrogens play a fundamental role in the development and normal physiological function of multiple tissue systems and have been implicated in the ontogeny of cancers. The biological effects of estrogens are classically mediated via interaction with cognate nuclear receptors. The relative expression of ER subtypes/variants varies between cells within different tissues and this alters the response to natural and synthetic ligands. This review focuses on the role of estrogen and estrogen related receptors in reproductive tissues.

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Contents

1. Background ...................................................................................................... 361
2. Structure of estrogen and estrogen-related receptors ........................................ 362
3. Estrogen receptor isoforms .............................................................................. 363
4. Molecular signaling pathways/functional interactions ....................................... 364
5. Estrogen receptor related proteins – functional activation and potential cross-talk with estrogen receptors ........................................................................... 365
6. Expression in the normal reproductive tissues and in reproductive cancers .... 365
6.1. Ovary ........................................................................................................... 365
6.2. Uterus ........................................................................................................ 366
6.3. Cervix ........................................................................................................... 366
6.4. Testis ........................................................................................................... 366
6.5. Efferent ductules, epididymis, vas deferens and seminal vesicles .................. 366
6.6. Prostate ....................................................................................................... 368
7. Evidence from rodent models ........................................................................... 368
7.1. Female reproductive system ........................................................................ 368
7.2. Male reproductive system ........................................................................... 368
7.3. Evidence for a functional role for ERRs in mice ........................................... 369
8. Future perspectives ......................................................................................... 369
References ............................................................................................................ 369

1. Background

Estrogens play a fundamental role in the development and normal physiological function of multiple tissue systems and have been implicated in the ontogeny of cancers. Estrogens are key regulators of fertility in both males and females. The biological effects of estrogens are classically mediated via their interaction with nuclear receptors that are members of a superfamily of ligand-activated transcription factors (http://www.nursa.org/). A recent review (Levin, 2010) has provided a comprehensive overview of the ways in which extra-nuclear receptors may also contribute to estrogen-dependent cell signaling and therefore this topic will not be discussed further in the current review.

The cDNA of the first estrogen receptor (ER) was cloned in 1986 (Green et al., 1986) and was thought to be the only estrogen receptor gene until a second ER was cloned a decade later (Kuiper et al., 1996). These receptors are known as ERα (NR3A1) and ERβ (NR3A2).
and are the products of two genes (ESR1 and ESR2, respectively) located in humans on chromosome 6q25.1 (ESR1) and 14q23.2 (ESR2). ERα and ERβ are members of the nuclear receptor subfamily 3 (NR3A); other members of the NR3 subfamily include the orphan estrogen related receptors (NR3B1–3; ERRα, ERRβ, ERRγ (Tremblay and Giguere, 2007) and the receptors for glucocorticoids (NR3C1, GR), mineralocorticoids (NR3C2, MR), progesterone (NR3C3, PR) and androgens (NR3C4, AR). Members of the NR3C subfamily are discussed in other reviews in this special issue. Evolutionary studies suggest that the ancestral steroid receptor was a functional estrogen receptor the sequence of which was conserved among descendant ERs (Thornton, 2001).

2. Structure of estrogen and estrogen-related receptors

ERs and ERRs, like other members of the NR3A family, contain a conserved arrangement of structural and functional domains (A–F) including a conserved DNA-binding domain (DBD, domain C) consisting of two zinc fingers and a C-terminal domain (domain E/F) that contains amino acids involved in ligand binding, receptor dimerisation and nuclear localization (reviewed in Tremblay and Giguere, 2007 and in protein–protein interactions and in transcriptional activation of target gene expression; it contains the activation function (AF)–1 region as well as several phosphorylation and sumoylation sites (Nilsson et al., 2001; Ascenzi et al., 2006) that are down stream targets for growth factor signaling pathways. Differences in the AF-1 domain may partially explain the distinctive responses of ERRs and ER to some ligands. For example, the anti-estrogens tamoxifen, raloxifene and ICI 182,780, or ERα alone e.g. MPP dihydrochloride, are available. Selective estrogen receptor modulators (SERMs) have agonist or antagonist properties depending on the cellular/tissue context (Bryant, 2001; Dutertre and Smith, 2000; Sun et al., 2002; Wakeling et al., 1991) (see Table 1).

The sequences within the N-terminal (A/B) domains of NR3A family members are poorly conserved with only 20% sequence homology between human ERα and ERβ. This domain is involved in protein–protein interactions and in transcriptional activation of target gene expression; it contains the activation function (AF)–1 region as well as several phosphorylation and sumoylation sites (Nilsson et al., 2001; Ascenzi et al., 2006) that are down stream targets for growth factor signaling pathways. Differences in the AF-1 domain may partially explain the distinctive responses of ERRs and ER to some ligands. For example, the anti-estrogens tamoxifen, raloxifene and ICI 182,780, or ERα alone e.g. MPP dihydrochloride, are available. Selective estrogen receptor modulators (SERMs) have agonist or antagonist properties depending on the cellular/tissue context (Bryant, 2001; Dutertre and Smith, 2000; Sun et al., 2002; Wakeling et al., 1991) (see Table 1).

The orphan receptor proteins ERRα, ERRβ and ERRγ share significant sequence homology with each other and also with ERα and ERβ. Although their putative ligand binding domains exhibit significant amino acid sequence identity with the ER LBDs the ERRs are unable to bind endogenous E2 because they lack the Cys residues involved in ligand recognition in ERα and ERγ. However the LBD in ERRα does contain an AF-2 domain that is the site for interaction with co-activators such as PPARY coactivator 1 (PGC1α or δ) or co-repressors such as RIP140 (Giguere, 2002). The LBD of ERRα is not functional but the ERRγ LBD is functional and has been postulated that modulation of ERR transcriptional activity is via natural antagonists (Giguere, 2002). Although natural ERR receptor modulators have not yet been identified synthetic compounds that can modulate the function of ERRs are available. For example, diethylstilbestrol (DES) can act as an inverse agonist on all three ERR isoforms (Giguere, 2002) and an inverse agonist called XCT 790 is available for ERRα (Busch et al., 2004). Synthetic agonists of ERRγ/ERRγ such as GSK 4716 and DY 131 have also been identified (see summary in Table 1).

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>Activity</th>
<th>Reference(s)</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β Estradiol (E2)</td>
<td>ERα, ERβ</td>
<td>Agonist</td>
<td>Gruber et al. (2002) and Kuiper et al. (1997)</td>
<td>Sigma, Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>ERα, ERβ</td>
<td>Agonist</td>
<td>Gruber et al. (2002) and Kuiper et al., 1997</td>
<td>Cayman Chemicals</td>
</tr>
<tr>
<td>PPT (propylpyrazole Triol)</td>
<td>ERα</td>
<td>Agonist</td>
<td>Harris et al. (2002), Kraichely et al. (2000) and Stauffer et al. (2000)</td>
<td>Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>DPN (diarylpropionitrile)</td>
<td>ER α&gt;&gt;&gt;ERα</td>
<td>Agonist</td>
<td>Meyers et al., 2001</td>
<td>Tocris, Sigma, Cayman Chemicals</td>
</tr>
<tr>
<td>WAY 200070</td>
<td>ER α&gt;&gt;&gt;ERα</td>
<td>Agonist</td>
<td>Malamas et al. (2004)</td>
<td>Tocris, Sigma</td>
</tr>
<tr>
<td>GSK4716 (GW4716)</td>
<td>ER α/β</td>
<td>Agonist</td>
<td>Zuecher et al., 2005</td>
<td>Sigma, Tocris</td>
</tr>
<tr>
<td>DY131</td>
<td>ERα/ERγ</td>
<td>Agonist</td>
<td>Yu and Forman (2005)</td>
<td>Sigma, Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>ERα/ERβ</td>
<td>SERM (mixed agonist/antagonist)</td>
<td>Dutertre and Smith (2000) and Kuiper et al. (1997)</td>
<td>Sigma, Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>Raloxifene (LY 139481, keoxifene)</td>
<td>ERα/ERβ</td>
<td>SERM (mixed agonist/antagonist)</td>
<td>Bryant, 2001; Dutertre and Smith (2000)</td>
<td>Sigma, Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>MPP dihydrochloride</td>
<td>ERα</td>
<td>Antagonist</td>
<td>Sun et al. (2002)</td>
<td>Tocris, Sigma</td>
</tr>
<tr>
<td>ICI 182,780 (Fastolde™)</td>
<td>ERα</td>
<td>Pure antagonist</td>
<td>Wakeling et al. (1991)</td>
<td>Sigma, Tocris, Cayman Chemicals</td>
</tr>
<tr>
<td>XCT790</td>
<td>ERα</td>
<td>Inverse agonist</td>
<td>Busch et al. (2004) and Lanvin et al. (2007)</td>
<td>Tocris, Sigma</td>
</tr>
</tbody>
</table>
3. Estrogen receptor isoforms

Ponglikitmongkol et al. (1988) reported that the human ERα gene is more than 140 kb in length and is split into eight exons. The human ERβ gene also contains 8 exons and there is considerable conservation between the arrangement of the coding exon/intron boundaries between human and mouse (Enmark et al., 1997).

Human ESR1 and ESR2 are both subject to alternative splicing and although they have similar exon and functional domain organization the splice variant isoforms identified appear to be distinct; as demonstrated by studies that have focused on expression of mRNA variants in cancer cell lines (Poola et al., 2002, 2000). Transcription of the ESR1 gene is initiated from multiple promoters resulting in mRNA isoforms with distinct 5’-untranslated regions (UTR); details of 7 of these are reviewed in Kos et al. (2002). The use of alternative promoters has been implicated in tissue specific patterns of expression and transcriptional regulation (Kos et al., 2002). Truncated isoforms have also been identified (Fig. 1). For example a 46-kDa ERα generated from an internal ATG start codon lacks exon 1 and consequently the N-terminal AF-1 domain (Flouriot et al., 2000). ERα46 can heterodimerize with full length ERα and inhibit AF-1-dependent transcriptional activity (Flouriot et al., 2000). ERα36 is generated from a promoter located in the first intron and lacks both AF-1 and AF-2 domains (Wang et al., 2005). A number of mRNA isoforms with exon-skipping have also been identified in cell lines (Poola et al., 2000) and a recent paper reported differential expression of ESRαΔ5/6/7, ESRαΔ3/4/5 in pre- and post-menopausal endometrium (Springwald et al., 2010).

The ESR2 gene appears to be particularly prone to alternative splicing with multiple variants formed from use of alternative promoters, inclusion or exclusion of different exons which have been identified in multiple tissues and cell lines. Poola et al. (2002) identified 10 variant mRNA isoforms of the human ESR2 gene with deletions in various combinations of exons several of which were present in extracts prepared from human cell lines and tissues including the ovary. The human ERβ (hERβ) protein can be expressed as both long (59.2 kDa) and short (53.3 and 54.2 kDa) isoforms (Scobie et al., 2002); these proteins appear to be functionally equivalent in transfection studies (Hall and McDonnell, 1999). In 1998 Ogawa et al. (1998) identified a novel human ERβ isoform that they called ERβcx, which was shorter than the wild type protein and had a unique stretch of 26 amino acids at its C-terminus. They demonstrated that these amino acids and an alternative 3’UTR were encoded by a novel exon that was alternatively spliced in place of exon 8. In the same year Moore et al. (1998) identified three full length, and two shortened isoforms of the human ESR2
Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ERa</th>
<th>ERb</th>
<th>ERRa</th>
<th>ERRb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>++</td>
<td>++</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Granulosa</td>
<td>++</td>
<td>++</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Pre-antral</td>
<td>++</td>
<td>++</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Antral</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Theca</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Uterus</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Myometrium</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Endometrium</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Progestin</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Stroma</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Epithelium</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Germ cell</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Testis</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Leydig</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Germ cell</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Prostate</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Stroma</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
<tr>
<td>Epithelium</td>
<td>+</td>
<td>+</td>
<td>Low</td>
<td>++</td>
</tr>
</tbody>
</table>

NDB not described. TBx 30-46 only in cancers. High ERb in ovary cancers. Staining is nuclear unless stated. Prostatic staining described as ‘weaker’ is relative to staining in other cell types within same tissue.

4. Molecular signaling pathways/functional interactions

The effects of estrogens can be mediated through several different pathways (reviewed in Nilsson et al., 2001; Hall et al., 2001; Matthews and Gustafsson, 2003). Classically, ligand-activated ERs form homo- or hetero-dimers that interact with response elements (called EREs, estrogen response elements) within the promoter regions of genes (reviewed in Nilsson et al., 2001). Gene transfer and band shift assays were originally used to identify an ERE consensus binding site within the promoter region of the vitellogenin gene of Xenopus laevis (Klein-Hitpass et al., 1989) this sequence was shown to consist of a palindromic repeat 5’-GATCTAGGTCACAGTGACCTA-3’. It has been reported that the binding affinity of ERα-ERα homodimers and ERα-ERβ heterodimers for consensus EREs is higher than ERα-ERβ homodimers (Cowley et al., 1997). Subsequent studies have established that estrogen receptors can also bind imperfect EREs and half sites, and can bind indirectly via other factors (reviewed in Nilsson et al., 2001; Welboren et al., 2007).
Key to our understanding of the range of ER binding sites has been the application of the method of chromatin immunoprecipitation (ChIP) in combination with genome-wide tiling arrays (ChIP-chip) or other methods (ChIP-PET, ChIP-seq). Most of these studies have used antibodies directed against ERα and have been performed using cancer cell lines such as MCF-7. They have revealed a potentially complicated regulation network with only a small portion of ERα binding sites being located in the promoter regions of known genes and many unforeseen binding sites a long distance from the putative transcription start site (Carroll et al., 2006). In a recent paper Gu et al. used informatics to reanalyze several of these datasets and they reported significant differences in the transcriptional networks in MCF-7 cells that were tamoxifen resistant (Gu et al., 2010). These studies have also revealed a role for other transcription factors in ER-dependent recruitment to EREs (reviewed in Welboren et al., 2007). Fewer studies have been conducted examining the ERβ-dependent transcriptome. In ERα-positive MCF-7 cells engineered with an inducible ERβ gene construct Li et al. (2008) found a high degree of overlap between the regions bound by ERα and ERβ, regions that were bound by ERα only in the presence of ERαβ, as well as regions that are bound by either receptor. Chang et al. (2008) used adenoviral delivery to engineer the relative expression of ERs. They reported that the phytoestrogen genistein preferentially activated ERαβ and proposed that differential occupancy of ERα and ERβ by genistein and E2 could influence recruitment patterns of co-regulatory proteins with knock-on consequences for patterns of gene expression.

Genomic studies have also revealed that only a third of the estrogen-responsive genes so far identified contain sequences in their promoters that resemble EREs (O’Lone et al., 2004). ER complexes can also bind through a variety of protein-protein interactions with transcription factors tethered to DNA such as Jun/Fos (at AP-1 response elements) or SP-1 (at GC-rich SP-1 motifs) or by interaction with the NFκ β pathway (De Bosscher et al., 2006).

ERs acting at different response elements may have agonist and antagonist profiles that differ from classical mechanisms of ER action; for example when tethered via AP-1 sites ERαβ exhibits E2-dependent activation of transcription at AP-1 sites, whereas E2 bound to ERβ has no effect (Paech et al., 1997). Both ERα and ERβ can interact with Sp transcription factors and while ERα-Sp1 complexes can be activated by estrogens little transcriptional activation is observed for Sp1-ERβ complexes. This may be due to AF-1 differences between the receptors (Saville et al., 2000). The antiestrogen ICI 182, 780 is a potent agonist to ERα and ERβ when they are tethered to AP-1, SP-1 and (O’Lone et al., 2004) STAT-5 transcription factors in the nucleus (Bjornstrom and Sjoberg, 2005). The affinities of different cofactors for ERs can also be subtype specific, for example, thyroid receptor-associated protein 220 (TRAP220) interacts with both ER subtypes but has selective affinity for ERα (Kang et al., 2002). In the presence of E2 the co-activator SRC-1 can be recruited to either ER but in the presence of genistein it is more strongly recruited to ERβ (Routledge et al., 2000). The role of cofactors in nuclear receptor function is reviewed elsewhere in this issue.

To date our understanding of the potential impact of expression of ERβ splice variants on estrogen responsiveness has been limited to studies using in vitro reporter systems. Co-expression of ERβ1 with ERα can result in a concentration dependent reduction in ERα-mediated transcriptional activity (Paech et al., 1997; Liu et al., 2002). Leung et al. (2006) reported that the ability of ERβ1 to activate an ERE-luciferase reporter gene in the presence of E2 was enhanced by co-expression of ERβ2, β4 or β5. In other studies it was reported that ERβ2 shows preferential hetero-dimerisation with ERα rather than ERβ1, inhibiting ERα DNA binding and having a dominant-negative effect on ligand-dependent ERα reporter gene activity (Ogawa et al., 1998).

5. Estrogen receptor related proteins – functional activation and potential cross-talk with estrogen receptors

Binding site selection experiments have demonstrated that ERR γ binds to a response element (ERRRE) containing a single consensus half-site, TNAAGGCTA, as either a monomer or a dimer (Sladek et al., 1997). It has been reported that ERs and ERRs have the capacity for transcriptional cross-talk with E-dependent genes such as osteopontin (reviewed in Vanacker et al., 1999). More recently studies using genomic analyses of binding sites have reported that ERαx and ERβ display strict binding site specificity and maintain independent mechanisms of transcriptional activation (Deblois et al., 2009). One mechanism of action for ERRs is by interaction with other transcription factors including ERs. For example ERRγ is reported to repress NF-E2 Related Factor 2 (Nf2f) activity via the antioxidant response element (ARE) through physical interaction in a complex with Nf2f and consistent with this ERRγ appears to be able to alter the subcellular localization of Nf2f (Zhou et al., 2007). Isomers of ERRγ can also differentially impact on ERα-dependent gene expression and this may in part relate to direct interaction between the proteins (Bombail et al., 2010a). Surprisingly androgen responsive genes can be down-regulated by inhibition of ERαx suggesting further capacity for cross-talk (Teyssier et al., 2008). Although the diverse functions of ERRs are yet to be fully explored in the reproductive system it is notable that a recent review claimed they are master regulators of mitochondrial biogenesis and function (Eichner and Giguere, 2011).

6. Expression in the normal reproductive tissues and in reproductive cancers

Estrogen receptors are widely expressed in reproductive tissues and data details are given in the following sections and summarised in Table 2.

6.1. Ovary

The ovary is the most important site of biosynthesis of estrogens in non-pregnant women of reproductive age. Expression of the aromatase enzyme complex, responsible for the conversion of theca-derived androgens, is up-regulated in mural granulosa cells of mature, antral follicles and also expressed in cells within the corpus luteum (Turner et al., 2002). Immunohistochemical studies in rodents (Saunders et al., 1997), primates and human (Saunders et al., 2000) have documented expression of ERα in all somatic cell types with intense staining of granulosa cells in immature and mature (antral) follicles as well as in the theca, corpus luteum and ovarian surface epithelium. In human ovaries immunohistochemical studies of ERα appeared restricted to the surface epithelium, theca cells and granulosa cells of mature follicles (Saunders et al., 2000; Pelleter and El-Alfy, 2000). Messenger RNAs encoding hERj2 and hERj4 have been detected in isolated human granulosa cells and their respective proteins immunolocalized to the same cell type in immature follicles (Scobie et al., 2002).

The vast majority (~90%) of ovarian cancers arise from the surface epithelium of the ovary (epithelial cell type) with the remainder arising from the granulosa cell (~5%) or the germ cell (1–2%) (http://www.cancerhelp.org.uk/type/ovarian-cancer/about/types-of-ovarian-cancer). Local biosynthesis of estrogen may occur in ovarian cancers as aromatase is reported to be expressed in up to ~80% of ovarian cancers. According to Li et al. (2008) in patients with recurrent ovarian cancer, for whom therapeutic options are limited, treatment with aromatase inhibitors has been shown to elicit clinical response rates of up to 35.7% and stable disease rates of 20–42% making them attractive options for treatment in this group of patients. In
Recent papers Halon et al. (2011) claimed that loss of ERβ expression in ovarian tumors may be a feature of malignant transformation and patients with a lower immunoreactivity score of ERα expression had significantly shorter overall survival time. Chu et al. (2000) demonstrated that expression of full length ERβ was higher in granulosa cell tumors than in those of epithelial origin and that ERβ2 (ERβcx) mRNA was widely expressed. In a subsequent paper they postulated that transcriptional resistance in ER-positive granulosa cell tumors was due to trans-repression by NFXβ (Chu et al., 2004).

Expression of ERRs mRNA has been detected in normal ovaries and is reportedly increased with clinical stage in ovarian cancers regardless of histopathological type; ERRβ and ERRγ mRNA expression was low in the same study (Fujimoto et al., 2007).

6.2. Uterus

The uterus has an outer muscular layer, the myometrium, which surrounds the endometrium that comprises a basal and functional layer. The functional layer of the endometrium undergoes cyclical growth, differentiation and regeneration under the influence of ovarian steroid hormones in the normal menstrual cycle and in the absence of pregnancy is shed during menses (Critchley and Saunders, 2009). Expression of ERα and ERRs is temporally and spatially regulated.

In the myometrium ERα and ERβ are both expressed but ERα mRNA expression is higher than ERβ (Jakimiuk et al., 2004). In the myometrium of postmenopausal women there is a switch in the relative expression of mRNAs with increased expression of ERβ and decreased expression of ERα compared to premenopausal myometrium. These changes are likely to be related to differences in bioavailable estrogens. During pregnancy there is a switch to predominance of ERβ over ERα in term myometrium (Sakaguchi et al., 2003).

In the normal pre-menopausal endometrium the ratio of ERα to ERβ changes according to the stage of the menstrual cycle. In the estrogen dominated proliferative phase, ERα expression is high in the glands and stroma (Critchley et al., 2001) (Fig. 2a) but ERα expression decreases in the secretory phase following the post-ovulatory rise in progesterone (Critchley et al., 2002). In Fallopian tube, ERβ mRNA is not down-regulated by peak progesterone levels and expression remains constant throughout the menstrual cycle (Horne et al., 2009). ERβ1 mRNA and protein are expressed throughout the cycle (Fig. 2b); ERβ2 is higher in the proliferative phase and is selectively down-regulated in the glandular epithelium during the secretory phase. In the uterus both ERα and ERβ proteins are expressed in multiple cell types, including the stroma and epithelial cells. ERβ but not ERα can be detected in endothelial cells that line blood vessel walls (Critchley et al., 2001) (Fig. 2c) and in immune cells such as uterine-specific natural killer (uNK) cells (Fig. 2d and e) (Henderson et al., 2003). In decidua of early pregnancy ERβ expression is minimal and restricted to nuclei of stromal and epithelial cells but nuclear expression of ERα is found in all compartments (Mline et al., 2005).

ERRs are also expressed in the uterus (Table 2). ERRα protein is expressed in the epithelial cells of glands, in stromal cells and endothelial cells at all stages of the cycle (Fig. 2f). A recent study reported increased expression of ERRα associated with decidualization of endometrial stromal cells in vitro; XCT790, an ERRα inverse agonist, inhibited expression of both ERRα and decidualisation markers such as IGFBP1 (Bombail et al., 2010b). ERRβ is expressed in human placenta (Fig. 2g) and has been localized to cell nuclei within the glands, stroma, endothelium and immune cells of the human endometrium throughout the cycle (Fig. 2h). In macrophages, uNK cells and endothelial cells ERRβ was co-expressed with ERβ. In epithelial cells during the proliferative phase ERRβ was co-expressed with ERα (Bombail et al., 2008). ERRγ has not been described in the uterus.

Endometrial cancer is the most common gynaecological malignancy. Greatest risk of developing the disorder is associated with factors related to excess exposure to estrogen, unopposed by progesterone, and a pro-inflammatory environment (Wallace et al., 2010). In a recent study Collins et al. (2009) reported that expression of ERα was reduced in poorly differentiated grade l cancers compared to those graded as well or moderately differentiated adenocarcinomas. In the same tissue set mRNAs encoding ERβ1, ERβ2 and ERβ5 did not vary significantly according to grade. The same isoforms were localized to cell nuclei in both the epithelial and stromal compartments with intense immunoeexpression of ERβ5 regardless of grade (Collins et al., 2009). In a separate study by Fujimoto and Sato (2009) the authors reported that a decrease in expression of both ERα and ERβ mRNAs correlated with a more advanced clinical stage, myometrial invasion and de-differentiation. In the same sample set expression of ERRα mRNA increased with clinical stage and myometrial invasion regardless of differentiation (Fujimoto and Sato, 2009).

6.3. Cervix

Expression of both ERα and ERβ has been described in stratal and epithelial cells, and in glandular epithelium of the cervix (Taylor and Al-Azzawi, 2000). Cervical vascular endothelium and cervical leukocytes express only ERβ (Stygar et al., 2001). During pregnancy the human cervix undergoes tissue remodeling in preparation for parturition which is associated with an increase in ERβ at term compared to non-pregnant cervix (Wang et al., 2001). Cervical cancer is often ERα positive and ERβ is required for carcinogenic activities of estrogen in the cervix (Chung et al., 2010). However carcinoma in situ of the cervix has been shown not to express ER (Chaudhuri et al., 1992). Studies in HPV transgenic mouse models suggest estrogen in combination with HPV oncopgenes may promote cervical cancer (Chung et al., 2008).

6.4. Testis

Immunoeexpression of ERα has not been detected in the adult human testis (Saunders et al., 2002; Makinen et al., 2001). Messenger RNA to multiple ERβ isoforms (ERβ1–5) have been isolated from cDNA pools prepared from human testis (Moore et al., 1998); ERβ2 and ERβ4 mRNA are reported to be expressed in germ cell enriched cell fractions (Aschim et al., 2004). Immunoeexpression of ERβ1 (Fig. 2i), and ERβ2 has been documented in distinct cell populations (Saunders et al., 2002). For example, immunostaining for ERβ1 was intense in pachytyne spermatocytes and round spermatids but lower in Sertoli cells, spermatogonia and preleptotene, leptotene, zygotene and diplotene spermatocytes. Intense immunoeexpression of ERβ2 has been described in Sertoli cells and spermatogonia but is variable in preleptotene, pachytyne, and diplotene spermatocytes. Low and variable expression of both ERβ1 and ERβ2 was detected in peritubular myoid and Leydig cells (Saunders et al., 2002). In isolated human germ cells the ERα46 protein has been detected in spermatozoa (Carreau et al., 2006).

In mice ERβ was expressed in seminiferous tubules and in adults is restricted to cells corresponding to spermocytes (Vanacker et al., 1998).

6.5. Efferent ductules, epididymis, vas deferens and seminal vesicles

In the male urogenital system of rodents and man the highest levels of expression of ERα have been detected in the efferent ductules that connect the testis to the head of the epididymis (Atanassova et al., 2001; Saunders et al., 2001). Expression of ERα in
Fig. 2. Immunolocalisation of ERs and ERRs in reproductive tissues. (a) ERα, human endometrium from proliferative phase, note intense staining of nuclei in endometrial glandular epithelium (G); (b) ERβ, human endometrium from secretory phase with immunopositive cells in glandular (G) and luminal epithelium as well as within the stromal compartment, blood vessel circled; (c) High power view of endometrial blood vessel showing ERβ positive endothelial cells (arrowhead); (d and e) double fluorescent immunostaining for uterine natural killer cells (CD56+, red, surface marker) in combination with ERα (d, green) or ERβ (e, green), note that CD56 positive cells are only immunopositive for ERβ (arrows in panel e); (f) ERRα, human endometrium. Note immunopositive staining of nuclei of cells lining the glands (G), stromal fibroblasts (S) and endothelial cells (arrowhead); (g) ERRβ, human placenta, first trimester. Immunopositive cell nuclei were detected in the cytotrophoblast cells but not in syncytiotrophoblast; (h) ERRβ, human endometrium, late secretory phase. The protein was expressed in nuclei of cells lining the glands (G), within the stroma and also both endothelial and perivascular cells lining blood vessels (rings); (i) ERRβ, adult human testis. Immunopositive cell nuclei were detected in both interstitial (Leydig cells, LC and blood vessels, BV) as well as within multiple cell types within the seminiferous tubules (ST) including Sertoli cells. Although intense immunopositive staining was detected in germ cells including pachytene spermatocytes and round spermatids (arrowheads) some pre-meiotic germ cells were immunonegative (open arrow); (j) ERα, human prostate. Immunopositive staining was detected in basal cells; (k) ERβ, human prostate. Protein was widely expressed in cells lining the glandular epithelium (E) and also in the stroma (S).
the epididymis is more variable and species specific. In human and primate expression of ERα was rarely detected in epithelial, basal cells or stromal cells but was more frequent in stromal cells of seminal vesicles. In contrast ERβ was detected in epithelial and stromal cell nuclei throughout the male reproductive system including the efferent ductules, epididymis, vas deferens and seminal vesicles (Saunders et al., 2001). ERβ2 and ERβ4 are both expressed in epithelial cells lining the vas deferens (Scobie et al., 2002).

6.6. Prostate

Estrogens have a significant effect on the development and homeostasis of the prostate gland and may have an important influence in prostatic diseases (reviewed in Ellem and Risbridger, 2010). ERα is predominantly expressed in the stromal compartment (Fig. 2). A study by Cheung et al. examined expression of ERs and ERRs in a range of prostate cell lines. ERα was weakly expressed in normal prostate epithelial cells, strongly expressed in the immortalized cell line RWPE-1, moderately expressed in the prostate cancer cell line PC3, but not in LNCaP or DU145 cell lines (Cheung et al., 2005).

ERβ is the dominant ER isof orm in the prostatic epithelium and is thought to inhibit proliferation and promote differentiation (Fig. 2) Prins and Korach, 2008. Leung et al. reported cytoplasmic staining of ERβ2 in basal and luminal epithelial cells and some nuclear staining in stromal cells; ERβ5 was strongly localized to basal epithelial cells and weakly stained some stromal cells (Leung et al., 2010). In cultured cells ERβ is reported to be low in normal prostate epithelial cells, moderate in the immortalized cell line RWPE-1, and moderate to strong in the prostate cancer cell lines PC3, LNCaP and DU145 (Cheung et al., 2005).

A recent study by Miao et al. described high expression of ERα in WPMY-1, a human normal prostate stromal cell line. In this study increased expression of ERα in response to prostaglandin (PGE2) also contributed to local estradiol production by upregulating aromatase expression (Miao et al., 2010). Cheung et al. described strong expression of ERRs in prostate cell lines, for example, ERRβ was strongly expressed in normal prostate epithelial cells and RWPE-1 but was only weakly expressed in PC3 and DU145. ERRβγ was expressed strongly in RWPE-1, LNCaP and PC3 but only weakly in DU145 and normal prostate epithelial cells. (Cheung et al., 2005).

It has been claimed that development of prostate cancer is associated with decreased expression of ERβ but expression may be regained in metastasis. In prostate cancer ERβ2 and ERβ5 expression have prognostic value. In patient samples, nuclear expression of ERβ2 and cytoplasmic expression of ERβ5 are both associated with an increased risk of reduced survival. In the same study the authors reported that over-expression of ERβ2 or ERβ5 in PC-3 cells resulted in a more invasive cell phenotype (Leung et al., 2010). In another study Fujimura et al. reported higher expression of ERβ2 and lower expression of ERβ1 in malignant prostatic tissue compared to benign tissue (Fujimura et al., 2001).

7. Evidence from rodent models

7.1. Female reproductive system

The estrogen receptor α knockout (ERαKO) mouse has an ovarian phenotype that is characterized by cystic and hemorrhagic follicles as well as anovulation. Couse et al. (1999) showed this phenotype could be reversed by addition of exogenous gonadotropin concluding the ovarian phenotype was due to disruption of the HPA axis. It was noted that ERαKO mice had an inefficient ovulatory capacity that may suggest an intra-ovarian role for ERα (Couse et al., 1999). The ovaries of theca-specific estrogen receptor α knockout (ThERαKO) mice show erratic oestrus and infertility from 4 months of age. Such ovaries have fewer corpora lutea and more antral follicles and superovulation stimulated the release of fewer oocytes of poor quality compared to wild types (Lee et al., 2009). The ovaries of the ThERαKO displayed some signs of hemorrhagic cyst formation that was enhanced with gonadotropin treatment (Lee et al., 2009). These findings suggest that loss of ERα in the theca may contribute to the ovarian phenotype in the global ERαKO mouse.

Studies on ERβKO mice show that the ovaries have reduced ovarian efficiency characterized by more early atretic follicles and reduced number of corpora lutea compared to wild-type ovariies. Numbers and size of litters is reduced and superovulation of ERβKO resulted in reduced number of oocytes (Krege et al., 1998). Large antral follicles of ERα+/ERβKO and ERβKO adults are markedly deficient in granulosa cells (Dupont et al., 2000). It has also been reported that trans/re-differentiation of granulosa cells occurs in the double knockouts resulting in a ‘Sertoli cell-like’ phenotype (Couse et al., 1999). In the aromatase knockout mouse (ArKO) ovarian function is disrupted due to arrested follicular development at the antral stage rendering the mice infertile (reviewed in Drummond et al., 2002).

Further evidence of the specific role of ERs in ovarian function is evidenced from the use of isotype-selective ER agonists. Hegele-Hartung et al. used highly selective agonists to ERα (16αL2E2) and ERβ (8β-V2E) to investigate the impact on ovarian function in rats. Treatment with 8β-V2E stimulated early folliculogenesis, decreased follicular atresia, induction of ovarian gene expression and stimulation of late follicular growth accompanied by an increase in oocytes without having stimulatory effects on uterine growth. Conversely treatment with the ERα agonist 16αL2E2 had a little or no ovarian effect but stimulated uterine growth (Hegle-Hartung et al., 2004).

The uterus is a major target tissue for estrogen action. Female ERαKO mice exhibit uterine hypoplasia with uterine weights that are half that of wild types with sparse distribution of glands (Dupont et al., 2000; Lubahn et al., 1993). The decidual response is intact but there is implantation failure (Curtis Hewitt et al., 2002). A uterine epithelial cell-specific ERα knockout model has also been reported and the studies on this mouse have demonstrated that proliferation of epithelial cells is mediated by an ERα-dependent proliferative signal from the stroma (Winuthayanon et al., 2010). In ERαKO mice uterine weight is normal however loss of functional ERα results in reduced epithelial cell differentiation and the ERαKO females are hyper-responsive to the proliferative effects of E2 consistent with a role for ERα as a negative regulator of ERα mediated response in this tissue (Dupont et al., 2000).

7.2. Male reproductive system

Estrogen has an important, although poorly understood, physiological role in regulating male reproductive organs. Notably, local biosynthesis of estrogen occurs in the testis with expression of aromatase being detected in Leydig cells and some populations of germ cells (Turner et al., 2002); ArKO mice develop a late onset testicular phenotype that can be partially reversed by a diet rich in phytoestrogens (Robertson et al., 2002). ERαKO male mice have a normal testicular phenotype until puberty but thereafter begin to degenerate and become atrophic due to a failure of resorption of fluid by the cells lining the efferent ductules creating ‘back pressure’ within the testis resulting in collapse of the seminiferous epithelium (Eddy et al., 1996; Hess et al., 1997). Male mice with targeted deletions of ERβ appear to have a normal testicular phenotype (Krege et al., 1998; Dupont et al., 2000).
Estrogens exert both direct and indirect effects on prostatic tissues and changes in the androgen: estrogen ratio in aging men has been cited as a risk for development of prostatic disorders including benign prostatic hyperplasia and cancer (Ellem and Risbridger, 2010). Studies in mice have not revealed a prostatic phenotype in studies using tissue recombinations and treatments with an ER-specific agonist that stimulation of ERα elicits anti-proliferative responses in epithelium suggesting that selective stimulation of ERα could benefit prostate health and inhibit disease development/progression (McPherson et al., 2007).

7.3. Evidence for a functional role for ERs in mice

In adult mice ERα is expressed in the kidney, heart, brown adipose tissue and tumours that preferentially metastasise to bone (Glotzbach et al., 2010). Evidence that ERs is involved in energy homeostasis has come from studies in ERαKO mice demonstrating they have resistance to diet induced obesity, altered fat metabolism and adsorption (Luo et al., 2003). They also fail to maintain body temperature in response to cold and have altered response to cardiac overload (Huss et al., 2007). ERβ is expressed during a narrow developmental window in trophoblast progenitor cells and is thought to play a role in early placentation consistent with impaired placental function in ERαKO (Fujimoto et al., 2005). ERβ is expressed in the central nervous system and is essential in oxidative metabolism of the heart; mice with targeted disruption of Erσr suffer from heart abnormalities and die during the first week of life (Alaynick et al., 2007).

8. Future perspectives

Estrogen receptors play an essential role in the development and normal physiological function of reproductive and other tissues. The relative expression of both full length and variant isoforms of ERs varies between cells within different tissues and this, together with variations in ligand availability adds to the complexity of responses to natural and synthetic ligands. Cancers of reproductive tissues are often hormone responsive and this has prompted the development of therapies based on SERMS and estrogen receptor subtype specific agonists. The impact of ER variants on cell function remains poorly understood as does the relative importance of non-ERE dependent signaling in defining the ER 'transcriptome' and both these topics require further investigation. New insight into the relative contributions of ERs to disease susceptibility may also come from the increasing number of studies examining single nucleotide polymorphisms in their genes (Chen et al., 2007). There is an increasing body of evidence that ERs may play a role in regulation of cell metabolism (Eicher and Giguere, 2011) but the full impact of this class of nuclear receptor on steroid responsiveness will require further study.

References


