REVIEW

The importance of ERβ signalling in the ovary

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Abstract

This review examines the evidence for a central role of oestrogen receptor β (ERβ or ESR2 as listed in the MGI Database) in folliculogenesis and hence reproductive biology. Knockout mouse models have been a valuable resource in this respect. The ERβ-null mouse exhibits a granulosa cell phenotype associated with the partial arrest of folliculogenesis and ovulatory dysfunction. Phyto-oestrogens such as genistein, which preferentially activate ERβ, have been shown to alleviate the ovarian phenotype of the oestrogen-depleted aromatase knockout mouse. In normal adult mice, genistein has been shown to cause reproductive defects following neonatal administration. Studies of ovarian cancer have also informed the literature. A decline in ERβ levels in epithelial ovarian cancers has been hypothesised to be associated with severity of disease and prognosis. Whereas the abundant expression of ERβ in granulosa cell tumours (GCT) of the ovary and evidence that ERβ signalling is transrepressed by the nuclear factor-κB pathway in GCT cell lines suggest a pathogenetic role for ERβ in GCT. In recent years, studies into the impact of environmental oestrogens (either in the form of pesticides or plastics) on reproductive function have shown that ERβ-selective toxins cause reproductive dysfunction and impair fertility. It remains to be established as to what genes are regulated by ERβ in the ovary. Finally, ERβ has been shown to be regulated by gonadotrophins, the pituitary hormones mediating ovarian function.

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Introduction

The identification of the second oestrogen receptor (ERβ or ESR2 as listed in the MGI Database) in 1996 (Kuiper et al. 1996, Mosselman et al. 1996) reignited research into the action of oestrogen throughout the body. Knockout mouse models were developed that either eliminated one or both of the receptors (ERα (ESR1) and ERβ; Lubahn et al. 1993, Couse et al. 1997, 1999, 2000, Krege et al. 1998, Dupont et al. 2000) or prevented oestrogen production (aromatase knockout mouse, ArKO: Fisher et al. 1998, Britt et al. 2000). ERα and ERβ exhibit species-/tissue-/cell-specific localisation and levels of expression (Fig. 1). ERβ has been detected primarily in the ovary (granulosa cells, theca cells, corpora lutea (CL), and oocyte), colon, brain, Fallopian tube, lung, adipose, kidney, bone, heart, bladder, adrenal, testis, and prostate (Enmark et al. 1997, Grohe et al. 1997, Kuiper et al. 1997, Saunders et al. 1997, 2002, Taylor & Al-Azzawi 2000, Anwar et al. 2001, Bord et al. 2001, Bocca et al. 2008, van den Driesche et al. 2008, Morani et al. 2008, Weiser et al. 2008), while ERα has been found predominantly in testis, epididymis, ovary (granulosa cells and theca cells), mammary gland, brain (pituitary gland), adipose, bone, heart, and uterus (Grohe et al. 1997, Kuiper et al. 1997, Drummond et al. 1999, Pelletier et al. 2000, Taylor & Al-Azzawi 2000, Anwar et al. 2001, Bord et al. 2001, Morani et al. 2008, Weiser et al. 2008). Although there has been considerable interest, driven by the pharmaceutical industry, in the role of ERβ in bone, the cardiovascular system, and in inflammation (for a review of ERβ’s roles in non-reproductive tissues, see Harris (2007)), its role in the ovary remains to be determined.

Oestrogen plays a pivotal role as an intrafollicular modulator, stimulating granulosa cell proliferation and facilitating the differentiative actions of FSH and LH on these cells (Richards 1980). Granulosa cells produce oestrogens, principally oestradiol (E2) from androgens via aromatisation, in response to FSH. The contribution of each ER to mediating these effects is becoming clearer: ERβ appears to be more directly implicated in ovarian development than ERα (Lubahn et al. 1993, Krege et al. 1998). This review will address the actions of ERβ in the ovary and its importance for normal ovarian function.

Oestrogen receptors

Steroid receptors are composed of five domains (Fig. 2) denoted A–F (Mangelsdorf et al. 1995). The DNA-binding domain (domain C) is highly conserved between ERα and
ERβ with an amino acid identity of 95%, whereas the homology in the ligand-binding domain (domain E) is only 55% (Kuiper et al. 1996). By way of perspective, this level of identity is also seen between the ligand-binding domains of the androgen, glucocorticoid, mineralocorticoid, and progesterone receptors, and is associated with both unique and shared ligand binding. The N-terminal (domains A/B), hinge (domain D), and C-terminal regions (domain F) have the greatest sequence diversity (Kuiper et al. 1996).

Multiple isoforms of the ERβ subtype have now been described (Chu & Fuller 1997, Petersen et al. 1998, Matthews & Gustafsson 2003, Poola et al. 2005, Zhao et al. 2005, 2008), although it is not clear whether these forms are all biologically active. Chu & Fuller (1997) reported the existence of a 54 nucleotide insert (Fig. 3A) in the ligand-binding domain of rat ERβ. Termed ERβ2, this isoform, present only in rodents (Chu & Fuller 1997, Lu et al. 1998), acts as a dominant negative regulator of ERβ- and ERα-mediated transcription (Maruyama et al. 1998). While this isoform has not been detected in humans, shortened transcripts and alternatively spliced forms of ERβ have been reported in normal ovary and ovarian tumours (Maruyama et al. 1998, Moore et al. 1998, Ogawa et al. 1998). These forms designated ERβ1, ERβ2 (also known as ERβcx), ERβ3, ERβ4, and ERβ5 (Ogawa et al. 1998, Inoue et al. 2000, Poola et al. 2005) each produce a full-length transcript despite truncations at the 3’-end (Fig. 3B). Initially, it was thought that ERβ4 and ERβ5 existed only as truncated transcripts but this has proven not to be the case (Poola et al. 2002, 2005).

The affinity of ligands for the respective receptor subtypes and isoforms present in the rat differs (Petersen et al. 1998). Of the human isoforms, ERβ1 is the only one that has been shown to have full function (Leung et al. 2006). Homodimeric forms of ERβ2, ERβ4, and ERβ5 have no activity but monomers of these forms can heterodimerise with ERβ1 to enhance its activation (Leung et al. 2006), raising the possibility of tissue-specific dimerisation. The response to oestrogen in a given tissue is defined by the ER expressed and the matrix of ER-interacting proteins present within the cells. These coregulatory molecules may influence the response in both a ligand- and promoter-dependent context, which, in turn, may be influenced by other signalling pathways. Nuclear hormone receptors interact with coregulatory proteins, either coactivators, which enhance transcription, or corepressors, which repress transcription. ER contains two ‘activation functions’ (AF) (see Fig. 2) that interact with coactivators:

![Figure 1 Schematic representation of oestrogen receptor localisation in human tissues. The level of expression of each receptor is not indicated. Structures shown are not to scale.](image1)

![Figure 2 Schematic representation of human ERα and ERβ. The amino acid sequence is numbered according to published data by Katzenellenbogen et al. (2000). The steroid receptor domains are indicated.](image2)
Definitive information on the expression of the respective ER mRNAs and proteins in granulosa cells of different follicle sizes is limited. In situ hybridisation and RT-PCR studies in the rat indicate that there is more Erβ than Erα mRNA in the ovary, and further analysis revealed more Erβ2 than Erβ1 in ovarian RNA collected from post-natal rats (Drummond et al. 1999). mRNAs for Erα and Erβ1 and Erβ2 are present in granulosa cells of follicles with at most two to three layers of granulosa cells (Drummond et al. 1996, 1999), and ERβ1 and ERβ2 proteins are present in rat granulosa cells (Drummond et al. 1996, Byers et al. 1997, Sar & Welsch 1999). In the human and marmoset, ERβ protein is expressed by granulosa cells of all follicle types, whereas ERα is not expressed by follicles containing one to two layers of granulosa cells (Saunders et al. 2000).

A convergence between gonadotrophin signalling and ERβ-mediated transcription in the ovary has been noted, unlike ERα. Gonadotrophins are important regulators of ovarian function and it makes sense for them to regulate ERβ expression if indeed ERβ is important for ovarian function. The LH surge has been found to downregulate Erβ mRNA in the ovaries of rats and hamsters (Byers et al. 1997, Yang et al. 2002), and gonadotrophin-induced cofactor-4 (GIOT-4 or ZFP709 as listed in the MGI Database), which is induced by FSH, coactivated ERβ in granulosa cells (Kouzu-Fujita et al. 2009).

Transducing ERβ signals

ERβs mediate transcription as dimers. Both homodimers and heterodimers of the ER activate transcription of reporter gene constructs containing oestrogen response elements (EREs), although heterodimers form preferentially in the presence of E2 (Pettersson et al. 1997, Petersen et al. 1998). It has been suggested that ERβ activity is compromised in the absence of ERα (Couse et al. 1997), further supporting the heterodimer as the functional form of ER. Studies in other tissues suggest that ERβ may antagonise/oppose the effects of ERα, thereby serving to limit cellular proliferation, promote differentiation (luteinisation), and modulate apoptosis (atresia).

Although a biological role for ERβ2 has not yet been elucidated, the studies of Maruyama et al. (1998) suggest that ERβ2 may be a negative regulator of oestrogen action, given that it dose dependently suppressed ERα- and ERβ1-mediated transcriptional activation. Thus, the formation of dimers containing ERβ2 may well induce very different effects on gene expression relative to those induced by receptor dimers, which do not contain ERβ2. In human cell lines, ERα and ERβ have been shown to signal in different ways depending on the ligand and response element (ERE or AP1) involved (Paech et al. 1997). It has also been demonstrated that the coexpression of ERα and ERβ in HeLa cells led to the suppression of the ERα-mediated gene cyclin D1 (Liu et al. 2002). There are other instances where functional antagonism of ERα by ERβ has been demonstrated, notably in models of fat reduction and cellular proliferation (Weihua et al. 2001). Microarray analyses

Localisation and regulation of ERβ in the ovary

of bone and liver tissue from ER-null mice support the inhibitory action of ERβ on ERα-mediated transcriptional activity (Lindberg et al. 2003).

ERβ plays a direct role in follicle development being required for antrum formation and preovulatory follicle maturation (Hegele-Hartung et al. 2004, Emmen et al. 2005). Ovulatory defects have been linked with polymorphisms of human ERβ (Sundarraj et al. 2001). Haemorrhagic and cystic follicles of ERα and LHβCTP (these mice express elevated levels of LH in the absence of ERβ) transgenic mice require ERβ for development (Couse et al. 2004). Polyovular follicles were induced by both ERα and ERβ agonists in neonatal mice (Nakamura et al. 2008). However, mice lacking ERβ do not produce polyovular follicles when challenged with genistein or diethylstilboestrol (DES) (Jefferson et al. 2002, Kim et al. 2009a), whereas ERα deplete mice do, suggesting that ERβ is directly involved in polyovular follicle formation. In human CL, oestrogenic activity is mediated by ERβ with both protein and mRNA localised to luteal cells, perivascular cells, and fibroblasts within the CL (Hosokawa et al. 2001). ERβ1 and ERβ2 mRNAs were differentially expressed across the luteal phase with ERβ1 maximally expressed in the mid-luteal phase and ERβ2 maximally expressed in the early luteal phase (van den Driesche et al. 2008). Colocalisation of the two forms was noted but not obligatory.

**ERβ-regulated genes**

Studies to identify genes regulated by ERβ in normal tissues have yet to be published; the few undertaken to date have utilised cancer lines (see Zhao et al. (2008) for a summary). Chang et al. (2006) investigated the effect of ERβ on gene regulation by MCF-7 cells expressing ERα. Microarray analyses revealed that genes regulating signal transduction pathways, cell cycle progression, and apoptosis were modulated by ERβ. These included members of the transforming growth factor–β superfamily (which are normally associated with suppression of breast cancer cell growth), class 3 and 4 semaphorin pathways, member of the forkhead box transcription factor family, only expressed in proliferating cells (FOXM1), cell division cycle 25 homologue A (CDC25A), transcription factor (E2F1), Survivin (member of the inhibitor of apoptosis protein family that acts as a suppressor of apoptosis and plays a central role in cell division), and cyclin–dependent kinase inhibitor (p21WAF1; Chang et al. 2006). Proliferation of MCF-7 cells declined when ERβ was present, consistent with the repression of FOXM1, CDC25A, E2F1, and Survivin mRNAs and the upregulation of p21WAF1, an inhibitor of cell proliferation and SEMA3B, a tumour suppressor (Chang et al. 2006).

In the presence of E2, ERβ enhanced the repression of Thrombospondin 1 (THBS1), reduced the repression of Integrin-6 and BMP-7, and downregulated stromal cell-derived factor–1 (SDF–1 or CXCL12; Chang et al. 2006), which has previously been shown to act as an autocrine growth factor for breast cancer cells (Hall & Korach 2003, Chang et al. 2006). Interestingly, SDF–1 has also been shown to interfere with semaphorin signalling. What is clear from these recent studies is that it is the relative levels of ERβ and ERα in a cell line/tissue which will determine the response to oestrogen.

**ERβ knockout mice**

Despite normal levels of gonadotrophins and ovaries that contain follicles of all stages of development and CL, ERβ knockout mice (BERKO) are subfertile producing fewer pups and litters and the ovaries yield fewer oocytes following superovulation (Krege et al. 1998, Couse et al. 2000, 2003, 2005, Dupont et al. 2000). A granulosa cell–specific phenotype of the BERKO mouse is evident (Couse et al. 2000). Ovaries of BERKO contain fewer large antral follicles and CL, and apoptosis in large follicles is increased (Emmen et al. 2005). It is clear that ERβ is important for follicle maturation from the antral stage of development and follicle rupture but not luteinisation (Emmen et al. 2005). The expression of genes important for ovarian differentiation is altered with reduced expression of aromatase, (Cyp19a1) LH receptor, (Lhgo) and prostaglandin–synthase 2 (Pgs2) mRNAs (Emmen et al. 2005) and increased expression of the androgen receptor in antral follicles (Cheng et al. 2002). Follicles from these mice produce significantly less E2 compared to wild type in vitro indicating an attenuated response to FSH (Couse et al. 2005).

It is apparent from the ERα knockout (ERKO) and BERKO ovarian phenotypes that ERα and ERβ have different roles to play in folliculogenesis. It has been hypothesised that the proliferative action of oestrogen is transmitted preferentially via ERα, whereas the differentiative effects of oestrogen are mediated principally by ERβ (Britt & Findlay 2002). This hypothesis is supported by the differentiation of granulosa cells into male-type Sertoli cells in the oestrogen–deficient, aromatase knockout (ArKO) (Britt et al. 2002). These Sertoli cells disappear from the ovaries of mice treated with E2 or phyto-oestrogens, principally genistein (Britt et al. 2002), an ERβ-selective ligand (Pettersson et al. 1997). However, interpreting the consequences of ERα and ERβ deletion in these models is complicated by the inability of these receptors to form heterodimers of ERα and ERβ. Homodimers of these transcription factors may induce very different effects on gene expression compared with ER heterodimers.

**Ovarian cancer**

The majority of ovarian cancers are epithelial in origin. Preliminary studies suggest that ERβ levels (protein and mRNA) in epithelial ovarian cancer decline relative to levels in normal ovary (Li et al. 2003, Bardin et al. 2004, Lindgren et al. 2004, Lazennec 2006). Overexpressing ERβ in an ovarian adenocarcinoma cancer cell line PEO14 led to a 50%
reduction in proliferative capacity (Lindgren et al. 2004). The prognostic significance of ER expression by ovarian cancers has received little attention although one study reported a correlation between levels of ERβ expression and cancer disease stage, with levels declining with increased severity of disease (Chan et al. 2008). In addition, breast cancer studies indicate that tumours positive for ERβ respond better to endocrine therapy (Fox et al. 2008). Thus, loss of ERβ expression may be a feature of malignant transformation.

An antimitotical role of ERβ in SK-OV-3 ovarian cancer cells that do not express functional ERα has been reported (Treeck et al. 2007). Reduced proliferation, inhibited motility, and increased apoptosis of SK-OV-3 cells overexpressing ERβ1 were noted. Exon-deleted ERβ1 splice variants ERβ–Δ125 and ERβ–Δ1256, which lack the AF-1 domain and have deletions in their DNA and ligand-binding domains, had no effect on proliferation or apoptosis but partly inhibited motility of these cells (Treeck et al. 2007). Genes associated with these physiological changes include an increase in p21 (WAF1), a cell cycle inhibitor, downregulation of cyclin A2, an oestrogen-responsive cell cycle regulator (Vendrell et al. 2004), and an increase in fibulin-1c, an extracellular matrix protein overexpressed in epithelial ovarian cancers and involved in motility (Hayashido et al. 1998). ERβ expression may be reduced as a result of DNA methylation (Zhao et al. 2008). Studies investigating epithelial ovarian carcinoma revealed that human promoter 0N was significantly methylated in ovarian cancer cell lines and tissues, and that this methylation correlated with decreases in the expression of exon 0N, ERβ1, ERβ2, and ERβ4 (Suzuki et al. 2008).

Granulosa cell tumours (GCT) account for about 5% of all ovarian cancers. GCT and GCT-derived cell lines abundantly express ERβ (Chu et al. 2000, Fuller et al. 2002, Fuller & Chu 2004), and their molecular phenotype is similar to preovulatory granulosa cells. As in other endocrine tumours, ERβ may be of pathogenetic significance. The steroid receptor coactivators SRC-1, –2, and –3 and the corepressors NcoR and SMRT are also expressed by GCT (Fuller et al. 2002). Despite ERβ expression and E2 binding, when GCT cell lines were transfected with oestrogen-responsive reporter genes and treated with E2, there was no response (Chu et al. 2004). The activation state of several signalling pathways in these lines was examined with both nuclear factor-κB (NFκB or NFKB) and AP-1 signalling found to be constitutively active. When the NFκB activity is inhibited by BAY 11–7082, ligand-dependent steroid receptor-mediated transactivation occurs for both exogenous and endogenous ERβ (Chu et al. 2004). Thus, ERβ signalling in GCT cell lines is transrepressed via the NFκB pathway.

Few studies have examined NFκB signalling in normal granulosa cells. We have localised p65 (RelA), a member of the NFκB family to granulosa cells, theca cells, oocytes, and luteal cells of adult rat ovary with both cytoplasmic and nuclear staining evident. Wang et al. (2002) reported that the NFκB pathway mediates the FSH-induced expression of X-linked inhibitor of apoptosis by granulosa cells. These data are consistent with a role for NFκB signalling in granulosa cells and indicate that ERβ signalling may be modulated by NFκB, perhaps through mutual transrepression. In malignant granulosa cells, inhibition of ERβ signalling by NFκB may be enhanced by cyclin D2 (Chu et al. 2004). Together, these data suggest that in both normal granulosa cell proliferation and in malignancy (GCT), the action of ERβ is inhibited by pro-proliferative signalling pathways, arguing that its role may be primarily to inhibit proliferation and/or promote differentiation. In GCT, this may contribute to the pathogenesis by interrupting part of an autocrine loop that contributes to limiting the FSH-like growth stimulation (Schumer & Cannistra 2003).

Environmental oestrogens

Ovarian–derived oestrogens are not the only compounds that can activate ER. Phyto-oestrogens are plant compounds with intrinsic oestrogen-like biological activity mainly due to the presence of a phenolic A ring, which is crucial for receptor binding (Bzozowski et al. 1997, Kuiper et al. 1998). There are two major classes of phyto-oestrogens: lignans and isoflavones. Soya protein contains the isoflavones, genistein and daidzein (Price & Fenwick 1985). Phyto-oestrogens are believed to signal predominantly via ERβ (Kuiper et al. 1997, Whitten & Naftolin 1998), and genistein, in particular, has a 20-fold higher binding affinity for ERβ compared to ERα (Kuiper et al. 1997, Whitten & Patiasul 2001, Martin et al. 2004). Feeding oestrogen-depleted ArKO mice diets containing either soy or genistein in part ameliorated the reproductive phenotype of female mice (Britt et al. 2005). Ovarian and uterine weights increased, although not to wild-type levels and haemorrhagic cysts disappeared with the addition of genistein. These effects of genistein are thought to be mediated via ERβ and are supported by the identification of ERβ in the uterus (Saunders et al. 1997, Matsuzaki et al. 1999, Sasano et al. 1999, Wang et al. 1999) and evidence that oestrogen directly (and not elevated LH) is responsible for the development of haemorrhagic cysts (Couse et al. 2004, Britt et al. 2005). Adverse effects of genistein on rodent reproductive function have also been reported (Jefferson et al. 2002, 2007b), notably reduced fertility, the formation of polyovular follicles, and altered oestrous cycles. The doses of genistein given neonatally to mice in these studies while high were environmentally relevant and led to the manifestation of reproductive abnormalities in adult life (Jefferson et al. 2007a).

Exposure of adult females to oestrogen either via the environment or clinically can have consequences for reproductive function. Adult rats treated with E2 valerate had abnormal oestrous cycles, while the ovaries contained reduced numbers of CL, developed follicular cysts, theca cell hyperplasia, and had an increase in apoptosis of granulosa cells from primary and secondary follicles (Shirwalkar et al. 2007). ERβ and PR proteins expressed by granulosa cells declined in...
follicles larger than secondary follicles suggesting abnormal differentiation of the granulosa cells.

Women exposed to endocrine-disrupting chemicals have impaired fertility and irregular menstrual cycles, and experience pregnancy loss (Fuortes et al. 1989). ERβ expression declined (no effect on ERα), and there were reduced expression of LHR and P450SCC mRNAs. Accelerated entry into puberty and to first oestrus, irregular cyclicity and reduced litter sizes have also been reported (Armenti et al. 2008). The bisphenol demethylated form HPTE (2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane) is believed to be responsible for the oestrogenic activity of MXC (Waters et al. 2001). HPTE analogues act as ERα agonists and ERβ antagonists in a range of cell lines (Gaido et al. 2000, Safe et al. 2001). ERβ was found to be hypermethylated, i.e. inactivated, whereas ERα was not (Zama & Uzumcu 2009).

Bisphenol A (BPA) exposure results from interactions with polycarbonate plastics or epoxy resins in food packaging (Kang et al. 2007). BPA acts as an agonist of oestrogen via ERβ, whereas it acts as both an agonist and antagonist in some cell types via ERα. The effect of BPA is likely to be determined on a tissue-specific basis (Safe et al. 2001, Kurosawa et al. 2002). Neonatal exposure to DES or BPA induces anovulation and persistent oestrus in female rodents (Iguchi 1992, Kato et al. 2003, Nakamura et al. 2008) and induces polyovular follicles (Kim et al. 2009b). The observed anovulation and induced oestrus are thought to be mediated via ERα, given that diarylpropionitrile, an ERβ-selective agonist, had no effect on these parameters (Nakamura et al. 2008).

Resveratrol (RES), a phyto-oestrogen found in grapes, binds equally to ERα and ERβ (Henry & Witt 2002). RES decreased body weight and induced ovarian hypertrophy potentially via ERβ in gonadally intact rats. RES-ligated ERβ induced significantly higher levels of transcriptional activity than E2-ligated ERβ, suggesting that tissues expressing ERβ will be more transcriptionally active in response to RES than those expressing ERα (Bowers et al. 2000).

Conclusion

ERβ still remains the most poorly understood of all steroid receptors despite more than 10 years elapsing since its identification. The studies reviewed here provide evidence for a role of ERβ in ovarian function, although the story is far from complete. Further studies are required to elaborate on the role of coactivators and corepressors in ERβ signalling, to elucidate the structure of the physiologically active dimer, and to identify genes specifically regulated by ERβ in the ovary.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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