Ovarian Actions of Estrogen Receptor-β: An Update

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Abstract

Estrogen is essential for folliculogenesis with independent roles attributed to each of the two estrogen receptors (ERs). ERβ, expressed predominantly by the ovarian granulosa cells, is required for antrum formation, preovulatory follicle maturation, expression of genes involved in ovarian differentiation (luteinizing hormone, aromatase, etc.), and follicle rupture during ovulation. Ovulatory dysfunction is associated with polymorphisms of the ERβ gene, and endocrine disruptors that selectively activate ERβ cause reproductive dysfunction and impairment fertility. ERβ may also exhibit antitumorigenic properties, with a decline in ERβ levels in epithelial ovarian cancers associated with more severe disease and poor prognosis. In this review, we examine the models that have been used to elucidate the roles ERβ plays in the ovary and consider the clinical consequences of altered ERβ expression or inappropriate activation of ERβ signaling.

Keywords

- ovary
- estrogen
- estrogen receptor
- cancer
- environmental estrogens

Estrogen is an essential intrafollicular modulator stimulating granulosa cell proliferation and facilitating the actions of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on ovarian cells.[1] In response to FSH, granulosa cells aromatize androgens to estrogens (primarily estradiol). The actions of estrogen are transduced by estrogen receptors (ERs) ERα and ERβ. ERβ was not identified until 1996,[2,3] at which point there was a resurgence of interest in estrogen action throughout the body. Knockout mouse models were developed that either eliminated one or both of the receptors (ERα and ERβ) or prevented estrogen production (aromatase knockout mouse [ArKO]).[4–11] ERα and ERβ exhibit specific tissue localization and levels of expression. ERβ is expressed in high levels in the ovary and prostate.[12] High levels of ERα are also present in the ovary, epididymis, testis, and pituitary.[12] Despite interest in the role of ERβ in bone, the cardiovascular system, and in inflammation (summarized in a review by Harris), its role in the ovary has received very little attention.[13] Here we review what is currently known about ERβ action in the ovary, in both health and disease states.

Estrogen Receptors

ERα and ERβ share 95% amino acid homology in the DNA binding domain and 55% homology in the ligand-binding domain (LBD).[2] This level of identity is also seen between the LBDs of the androgen, glucocorticoid, mineralocorticoid, and progesterone receptors (PRs), and it is associated with both unique and shared ligand binding. The N-terminal, hinge, and C-terminal regions of the ER have the greatest sequence diversity.[2]

Multiple isoforms of the ERβ subtype have now been described,[14–19] although it is not clear if these forms are all biologically active. Chu and colleagues reported the existence of a 54 nucleotide insert in the LBD of rat ERβ.[14] Termed ERβ2, this isoform, present only in rodents,[14,20] acts as a dominant negative regulator of ERβ- and ERα-mediated transcription.[21] Although this isoform has not been detected in humans, shortened transcripts and alternatively spliced forms of ERβ have been reported in normal ovary and ovarian tumors.[21–23] These forms, designated ERβ1, ERβ2 (also known as ERβ cx), ERβ3, ERβ4, and ERβ5,[17,23,24] each produce a full-length transcript. Initially it was thought that ERβ4 and

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ERβ5 existed only as truncated transcripts, but this has proven not to be the case.[17,25]

The affinity of ligands for the respective receptor subtypes and isoforms differs.[15] The response to estrogen in a given tissue is defined by the ER expressed and the matrix of ER-interacting proteins present within the cells. These co-regulatory molecules may influence the response in both a ligand- and promoter-dependent context, which in turn may be influenced by other signaling pathways. Nuclear hormone receptors interact with co-regulatory proteins, either coactivators that enhance transcription or corepressors that repress transcription. ERs contain two activation functions (AFs) that interact with coactivators. AF-1, which is ligand independent, lies within the N-terminal domain; AF-2 lies in the LBD, and its activity depends on ligand-induced conformational changes. The relative contribution of each AF is cell and promoter dependent. Transcription of the human ERβ genes occurs from at least two promoters, 0N and 0K,[19] with the same transcript produced.

Signaling Via Estrogen Receptor-β

ERs mediate transcription as dimers. Both homodimers and heterodimers of the ER activate transcription of reporter gene constructs containing estrogen response elements.[15,26] It has been suggested that ERβ activity is compromised in the absence of ERα,[4] further supporting the heterodimer as the functional form of ERs. Studies in other tissues suggest that ERβ may antagonize/oppose the effects of ERα, thereby serving to limit cellular proliferation, promote differentiation (luteinization), and modulate apoptosis (atresia).[27] Although a biological role for ERβ2 has not yet been elucidated, the studies of Maruyama et al suggested that ER-β2 may be a negative regulator of estrogen action, given that it dose dependently suppressed ERα and ERβ1-mediated transcriptional activation.[21] Thus the formation of dimers containing ERβ2 may well induce very different effects on gene expression relative to those induced by receptor dimers that do not contain ERβ2.

ERβ plays a direct role in follicle development and is required for antrum formation and preovulatory follicle maturation.[27,28] Ovulatory defects have been linked with polymorphisms of human ERβ.[29] Hemorrhagic and cystic follicles of ERα and LHβ C-terminal peptide transgenic mice (mice that express elevated levels of LH in the absence of ERβ) require ERβ for development.[30] Polyovular follicles were induced by both ERα and ERβ agonists in neonatal mice.[31] However, mice lacking ERβ do not produce polyovular follicles when challenged with genistein or diethylstilbestrol (DES),[32,33] whereas ERα knockout mice do, suggesting that ERβ is directly involved in polyovular follicle formation. In human corpora lutea (CL), estrogenic activity is mediated by ERβ with both protein and mRNA localized to luteal cells, perivascular cells, and fibroblasts within the CL.[34] ERβ1 and ERβ2 mRNAs were differentially expressed across the luteal phase with ERβ1 maximally expressed in the midluteal phase and ERβ2 maximally expressed in the early luteal phase.[35] Co-localization of the two forms was noted but not obligatory.

Localization and Regulation of Estrogen Receptor-β

ERβ is present in the ovaries of a wide number of species, including mouse, rat, rabbit, sheep, cow, baboon, hamster, pig, and human.[4,20,36–45] Whereas ERβ is predominantly expressed by the granulosa cells, theca cells, surface epithelium, and CL, although oocytes have also been reported to express the receptor.[34,36,39,46–50]

Definitive information on the expression of the respective ER mRNAs and proteins in granulosa cells of different follicle sizes is lacking. In situ hybridization and reverse transcriptase polymerase chain reaction studies in the rat indicate there is more ERβ than ERα mRNA in the ovary, and further analysis revealed more ERβ2 than ERβ1 in ovarian RNA collected from postnatal rats.[37] Messenger RNA transcripts for ERα and ERβ1 and ERβ2 are present in granulosa cells of follicles with at most two to three layers of granulosa cells, and ERβ1 and ERβ2 proteins are present in rat granulosa cells.[27,36,51,52]

A convergence between gonadotropin signaling and ERβ-mediated transcription in the ovary has been noted, unlike ERα. Gonadotropins are important regulators of ovarian function, and thus it makes sense for them to regulate ERβ expression if indeed ERβ is important for ovarian function. The LH surge was found to downregulate ERβ mRNA in the ovaries of rats and hamsters, and gonadotropin-induced cofactor-4 induced by FSH coactivated ERβ in granulosa cells.[36,38,53]

Genes Regulated by Estrogen Receptor-β

Studies to identify genes regulated by ERβ are difficult to find for normal tissues; the few undertaken to date have used cancer cell lines.[19] Chang and colleagues investigated the effect of ERβ on gene regulation by MCF-7 cells expressing ERα.[54] 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, FOXM1 (member of the forkhead box transcription factor family, only expressed in proliferating cells), CDC25A (cell division cycle 25 homologue A), E2F1 (transcription factor), 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 p21WAF1 (cyclin-dependent kinase inhibitor).[54] 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 tumor suppressor.[54]

In the presence of estradiol, ERβ enhanced the repression of thrombospondin 1, reduced the repression of integrin 6 and bone morphogenetic protein-7, and downregulated stromal cell derived factor (SDF)-1.[54,55] SDF-1, which has previously been shown to act as an autocrine growth factor for breast cancer cell, has also interestingly been shown to interfere with semaphoring signaling.[54,55] We are currently undertaking microarray analyses of our granulosa cell tumor cell lines and hope in the near future to report on genes

regulated by ERβ in reproductive cells. These recent studies make clear that it is the relative levels of ERβ and ERα in a cell line/tissue that will determine its response to estrogen.

Estrogen Receptor-β Knockout Mice

Despite normal levels of gonadotropins and ovaries that contain follicles of all stages of development and CL, ERβ knockout mice (βERKO) are subfertile, producing fewer pups and litters and yielding fewer oocytes following superovulation.[5,7,9,56,57] Investigators have suggested that a disruption in communication between the theca and granulosa cell layers leads to inhibition of vascularization, preventing the increase in permeability and hyperemia that facilitates expulsion of the ovum.[58] Wedge resection of ERβ knockout ovaries, with its presumed effects on improving vascularization, restored fertility to 100%.[58]

Furthermore, the βERKO mouse displays a granulosa cell–specific phenotype.[5] Ovaries of the βERKO mice contain fewer large antral follicles and CL, and apoptosis in large follicles is increased.[27] It is clear that ERβ is important for follicle maturation from the antral stage of development to follicle rupture.[27] ERβ also appears to play a role in the expression of genes that are important for ovarian differentiation, with βERKO mice demonstrating decreased aromatase, LH receptor, and prostaglandin synthase (Ptgs)2 mRNA levels and increased androgen receptor expression within antral follicles.[27,59] Follicles from these mice also produce significantly less estradiol compared with wild-type mice in vitro, indicating an attenuated response to FSH.[57] ERβ recently was shown to be required by preovulatory follicles for the production of cyclic adenosine monophosphate (cAMP), and inadequate levels of cAMP may account for the reduced levels of estradiol produced by these follicles.[60,61]

It is apparent from the ERα knockout (ERKO) and βERKO ovarian phenotypes that ERα and ERβ have different roles to play in folliculogenesis. It has been hypothesized that the proliferative action of estrogen is transmitted preferentially via ERα, whereas the differentiating effects of estrogen are mediated principally by ERβ.[62] This hypothesis is supported by the differentiation of granulosa cells into male-type Sertoli cells in the estrogen-deficient ArKO.[63] These Sertoli cells disappear from the ovaries of mice treated with estradiol or phytoestrogens, principally genistein,[63] an ERβ-selective ligand.[26] 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.

Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) is a common endocrine disorder characterized by anovulation, elevated levels of androgen, hirsutism, and insulin resistance.[64,65] Folliculogenesis is arrested at the antral stage of development, and it is the accumulation of these follicles that gives the ovary its characteristic morphology of a necklace-like pattern of follicles in the periphery. Because estrogen has been shown to be essential for folliculogenesis beyond the antral stage, it is perhaps not surprising that ERβ mRNA and protein are reduced in granulosa cells and theca cells from PCOS patients.[11,66] We hypothesized that changes in the ratio of ERβ το ERα may result in abnormal follicular development. Similarly, in a rodent model of PCOS, levels of ERβ protein were decreased in the granulosa layers of cystic follicles.[67] Idiopathic ovulatory dysfunction has been found to be associated with a G/A (1730) polymorphism in ERβ.[2]. Given that ovulatory dysfunction is a key feature of PCOS, one group investigated a cohort of PCOS patients to determine if there was an association with this polymorphism.[68] They reported significant differences in the genotype distribution and allelic frequencies between controls and PCOS patients that supported a correlation with the G/A polymorphism.[68] To date, the underlying mechanism has not been established.

Ovarian Cancer

Most 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.[69–72] Overexpressing ERβ in an ovarian adenocarcinoma cancer cell line PEO14 led to a 50% reduction in proliferative capacity.[70] 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.[73] In addition, breast cancer studies indicate that tumors positive for ERβ respond better to endocrine therapy.[74] Thus loss of ERβ expression may be a feature of malignant transformation.

An antitumoral role of ERβ in SK-OV-3 ovarian cancer cells that do not express functional ERα was reported.[75] Reduced proliferation, inhibited motility, and increased apoptosis of SK-OV-3 cells overexpressing ERβ1 were noted.[75] Exon-deleted ERβ1 splice variants ERβ-δ125 and ERβ-δ1256, which lack the AF-1 domain and have deletions in their DNA and LBDs, had no effect on proliferation or apoptosis but partly inhibited motility of these cells.[75] Genes associated with these physiological changes include an increase in p21 (WAF1), a cell cycle inhibitor, downregulation of cyclin A2, an estrogen-responsive cell cycle regulator, and an increase in fibullin-1c, an extracellular matrix protein overexpressed in epithelial ovarian cancers and involved in motility.[76,77] ERβ activity may be reduced as a result of DNA methylation.[19] 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.[78] Furthermore, treatment of ovarian cancer cells in vitro with demethylating agents has been shown to restore ERβ activity and inhibit growth, suggesting that ERβ activity is antitumorigenic.[79]

GCTs account for ~5% of all ovarian cancers. GCT and GCT-derived cell lines abundantly express ERβ, and their molecular phenotype is similar to preovulatory granulosa cells.[80–83]

As in other endocrine tumors, ERβ may be of pathogenetic significance. The steroid receptor coactivators SRC-1, -2 and -3 and the co-repressors NcoR and SMRT are also expressed by GCT.[81] Despite ERβ expression and estradiol binding, when GCT cell lines were transfected with estrogen-responsive reporter genes and treated with estradiol, there was no response.[84] The activation state of several signaling pathways in these lines was examined with both nuclear factor (NF)κB and AP-1 signaling 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.[84] Thus ERβ signaling in GCT cell lines is transrepressed via the NFκB pathway.

Few studies have examined NFκB signaling in normal granulosa cells. We have localized 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 reported that the NFκB pathway mediates the FSH-induced expression of X-linked inhibitor of apoptosis (XIAP) by granulosa cells.[85] These data are consistent with a role for NFκB signaling in granulosa cells and indicate that ERβ signaling may be modulated by NFκB, perhaps through mutual transrepression. In malignant granulosa cells, inhibition of ERβ signaling by NFκB may be enhanced by cyclin D2.[84] Together, these data suggest that in both normal granulosa cell proliferation and in malignancy (GCT), the action of ERβ is inhibited by pro-proliferative signaling 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.[86]

Environmental Estrogens

Ovarian-derived estrogens are not the only compounds that can activate ER. Phytoestrogens are plant compounds with intrinsic estrogen-like biological activity mainly due to the presence of a phenolic A ring, which is crucial for receptor binding.[87,88] The two major classes of phytoestrogens are lignans and isoflavones. Soy protein contains the isoflavones genistein and diadzein.[89] Phytoestrogens are believed to signal predominantly via ERβ, and genistein in particular has a 20-fold higher binding affinity for ERβ compared with ERα.[12,90–92] Feeding estrogen-depleted ArKO mice diets containing either soy or genistein in part ameliorated the reproductive phenotype of female mice.[93] Ovarian and uterine weights increased, although not to wild-type levels, and hemorrhagic cysts disappeared with the addition of genistein.[93] These effects of genistein are thought to be mediated via ERβ, which is supported by the identification of ERβ in the uterus and evidence that estrogen is directly responsible for the development of hemorrhagic cysts (and not elevated LH levels).[30,93–97] Adverse effects of genistein on rodent reproductive function have also been reported, notably reduced fertility, the formation of polyovular follicles, and altered estrous cycles.[32,98] The doses of genistein given neonatally to mice in these studies were high, although environmentally relevant, and led to the manifestation of reproductive abnormalities in adult life.[99]

Exposure of adult females to estrogen either via the environment or clinically can have consequences for reproductive function. Adult rats treated with estradiol valerate had abnormal estrus cycles, and the ovaries contained reduced numbers of CL, developed follicular cysts, and theca cell hyperplasia, and there was an increase in apoptosis of granulosa cells from primary and secondary follicles.[100] ERβ and PR proteins expressed by granulosa cells declined in follicles larger than secondary follicles, suggesting abnormal differentiation of the granulosa cells.[100]

Women exposed to endocrine-disrupting chemicals have impaired fertility, irregular menstrual cycles, and experience pregnancy loss.[101,102] Methoxychlor (MXC), an organochlorine pesticide with estrogenic activity mediated primarily via ERβ, caused ovarian dysfunction in the adult rodent following exposure to rats during the fetal or neonatal period.[12,103] Follicle composition was altered with more preantral and early antral follicles present and fewer CL.[104] ERβ expression declined, and there was reduced expression of LH receptor and P450SCC mRNAs. Accelerated entry into puberty and to first estrus, irregular cyclicity, and reduced litter sizes were also reported.[105] The bisphenol demethylated form HPTE is believed to be responsible for the estrogenic activity of MXC.[106] HPTE analogs act as ERα agonists and ERβ antagonists in a range of cell lines.[107,108] ERβ was found to be hypermethylated (i.e., inactivated), whereas ERα was not.[103]

Bisphenol A (BPA) exposure results from interactions with polycarbonate plastics or epoxy resins in food packaging.[109] BPA acts as an agonist of estrogen 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.[108,110] Neonatal exposure to DES or BPA induces anovulation and persistent estrus in female rodents and induces polyovular follicles.[31,111–113] The observed anovulation and induced estrus is thought to be mediated via ERα, given that diarylpropionitrile, an ERβ selective agonist, had no effect on these parameters.[31]

Resveratrol (RES), a phytoestrogen found in grapes, binds equally to ERα and ERβ.[114] RES decreased body weight and induced ovarian hypertrophy potentially via ERβ in gonadally intact rats. RES-liganded ERβ induced significantly higher levels of transcriptional activity than estradiol-liganded ERβ, suggesting that tissues expressing ERβ will be more transcriptionally active in response to RES than those expressing ERα.[115]

Conclusion

ERβ plays an essential role in ovarian function; changes in expression or activation of ERβ may have clinical consequences that take the form of infertility or cancer. Future studies need to elucidate the structure of the physiologically active dimer, identify genes specifically regulated by ERβ in the ovary, and address the role of coactivators and corepressors in ERβ signaling.

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