The 16th Datta Lecture[1]

Update on estrogen signaling

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Abstract Our understanding of estrogen signaling has undergone a true paradigm shift over recent years, following the discovery in 1995 of a second estrogen receptor, estrogen receptor β (ERβ). In many contexts ERβ appears to antagonize the actions of ERα (yin/yang relationship) although there also exist genes that are specifically regulated by one of the two receptors. Studies of ERβ knockout mice have shown that ERβ exerts important functions in the ovary, central nervous system, mammary gland, prostate gland, hematopoiesis, immune system, vessels and bone. The use of ERβ-specific ligands against certain forms of cancer represents one of the many pharmaceutical possibilities that have been created thanks to the discovery of ERβ.

Key words: Estrogen receptor; Estrogen; Breast cancer; Prostate; Central nervous system

1. Estrogen receptor β, a new member of the steroid hormone receptor family

Estrogen receptors (ER) belong to the steroid/retinoid receptor gene superfamily, the members of which share some structural and functional similarities [1]. There are four structurally distinct, functional domains in these proteins. From the N-terminus to the C-terminus of the receptor molecule these are: the N-terminal A/B region that contributes to transcriptional activation function and harbors the activation function 1 (AF-1); the mid-region also called the C region, or the DBD, since it harbors the DNA binding domain (Fig. 1A). This is the region that mediates specific DNA binding. Next to the DBD is the D domain or the hinge region, a less well-characterized region; at the C-terminus is the ligand binding domain (LBD or E/F domain). LBD harbors the ligand binding pocket as well as sites for cofactor binding, transactivation (AF-2), nuclear localization and interactions with heat shock proteins. Functionally, upon ligand binding, these receptors form dimers and modulate transcription by binding to their corresponding hormone response element (ERE for estrogen response element) in the promoter region of target genes.

The first estrogen receptor, now named ERα, was cloned in 1986 [2,3], more than 20 years after it was identified by its affinity for 17β-estradiol. In 1996, the second ER, ERβ, was reported [4]. A schematic comparison of ERβ and ERα is shown in Fig. 1. There is 96% amino acid identity between the two receptors in the DBD but in the LBD the homology is only 53%. This suggests that ERβ would recognize and bind to similar EREs as ERα but that each receptor would have a distinct spectrum of ligands. There is less conservation between the two receptors in the N-terminal AF-1 and C-terminal AF-2. This suggests that different sets of proteins in the transcription complexes may interact with ERα and ERβ and direct them to specific targets. Such a mechanism could explain why at AP-1 sites, ERβ and ERα function oppositely [5]. Experimental evidence has shown that both ERs do bind to classical EREs with similar affinities and that their affinities for 17β-estradiol (E2) are quite similar (0.6 nM for ERβ and 0.2 nM for ERα) [4,6]. However, many other ligands show preferential binding to one or the other ER. Some natural dietary components, coumestrol and genistein, show a preference for binding ERβ [7] and can probably be further modified to create α- and β-selective estrogen receptor modulators. The chemical structures of three ER ligands are shown in Fig. 2.

E2 target tissues can be divided into two groups, the classical and non-classical E2 target tissues, respectively. The classical targets are the uterus, mammary gland, placenta, liver, central nervous system (CNS), cardiovascular system, bone.

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Abbreviations: E2, 17β-estradiol; ER, estrogen receptor; DBD, DNA binding domain; LBD, ligand binding domain; AF, activation function; ERE, estrogen response element; ER−/−, estrogen receptor knockout; wt, wild type; SRC, steroid receptor coactivator; Ar, aromatase; DHT, 5α-dihydrotestosterone; 3βAdiol, 5α-androstane-3β,17β-diol; HSD, hydroxysteroid dehydrogenase; PR, progesterone receptor

[1] The previous Datta Lectures were given by: F. Melchers (1st, 1986); N. Sharon (2nd, FEBS Letters 217 (1987) 145–157); B.G. Malmström (3rd, FEBS Letters 250 (1989) 9–21); J.C. Skou (4th, FEBS Letters 268 (1990) 314–324); B.A. Lynch and D.E. Koshland, Jr. (5th, FEBS Letters 307 (1992) 3–9); A.R. Fersht (6th, FEBS Letters 325 (1993) 5–16); E. Sackmann (7th, FEBS Letters 346 (1994) 3–16); P. De Camilli (8th, FEBS Letters 369 (1995) 3–12); C. Weissmann (9th, FEBS Letters 389 (1996) 3–11); Ph. Cohen et al. (10th, FEBS Letters 410 (1997) 3–10); L.N. Johnson et al. (11th, FEBS Letters 430 (1998) 1–11); J.-M. Egly (14th, FEBS Letters 498 (2001) 124–128); the 12th, 13th and 15th Datta Lectures have not been published.

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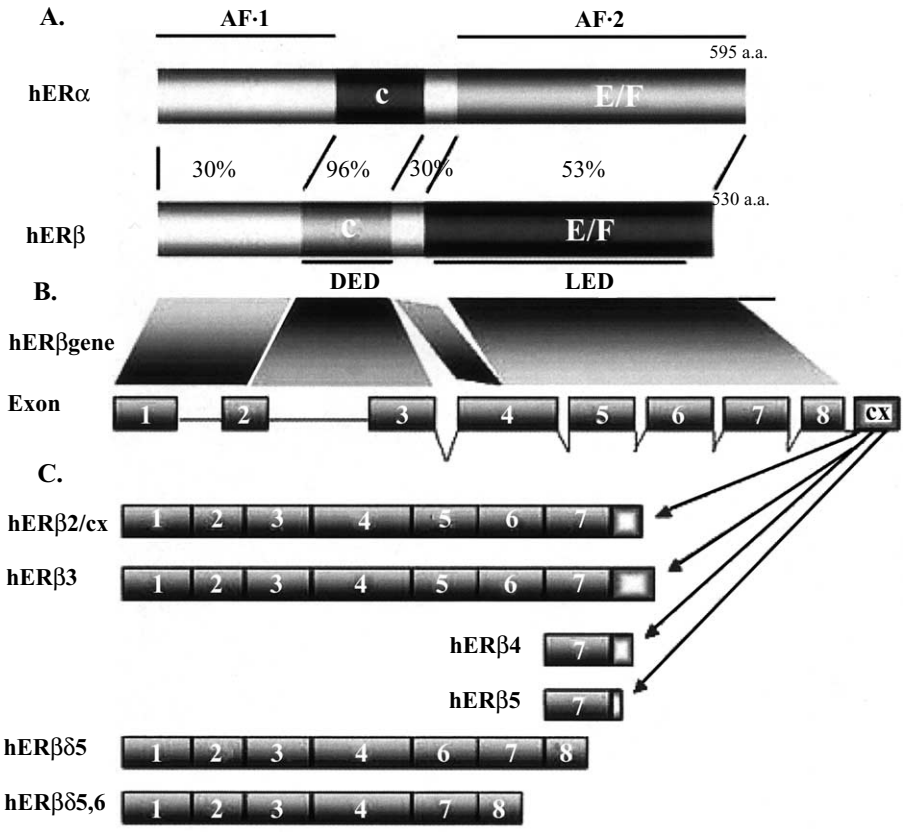

Fig. 1. Structural comparison of ERα, ERβ and ERβ splice variants. The percent homology in the various functional domains of the two receptors is given in A. In B the structural organization of the ERβ gene is illustrated. Exons 1–8 are utilized in ERβ1 but, as illustrated in C, in the splice variant ERβcx, exon 8 is replaced by the alternative exon, labeled cx.

These tissues have a high ERα content and respond to E2 challenge with increases in transcription of certain E2-responsive genes. The non-classical target tissues include prostate, testis, ovary, pineal gland, thyroid gland, parathyroids, adrenals, pancreas, gallbladder, skin, urinary tract, lymphoid and erythroid tissues. In these tissues, expression of ERα is either very low or not measurable. ERβ is highly expressed in non-classical E2 target tissues like the prostate epithelium, urogenital tract, ovarian follicles, lung, intestinal epithelium, certain ERα-deficient brain regions and muscle [8].

In mature females the major estrogen target tissue is the uterus. It contains very little ERβ but is abundant in ERα. It was from this tissue that Jensen and Jacobsen found specific binding of E2, and concluded that the biological effects of estrogen had to be mediated by a receptor protein [9]. In the breast, E2 stimulates growth and the estrogen receptor antagonist tamoxifen has been the most effective treatment for ERα-positive breast cancer [10]. The reason that ERβ was not found until 1995 is probably because the focus of estrogen studies had not been directed to tissues where ERβ is abundant. Although some studies explored estrogen actions in tissues like the ventral prostate, where ERα expression is low, interpretation of the data was always colored by the view that ERα was the only ER. It is possible that if the prostate, lung or granulosa cells had been used to clone ER, ERβ would have been the first ER to be found.

It is generally accepted that ER sediments at 8S when cytosol is extracted with low salt buffer (10 mM KCl) and at 4S when high salt buffer (100 mM KCl) is used [11]. When ERβ was cloned and expressed in insect cells, it was found that this new ER sediments at 4S regardless of the salt concentrations in the buffer [12–14]. Before 1995, there are some reports in the literature where 4S E2 binding peaks were found when tissues were analyzed [15–19]. These results were not understood but today we know that these are ERβ-containing tissues, and the 4S peak was probably ERβ. The reason for different sedimentation properties between ERα and ERβ

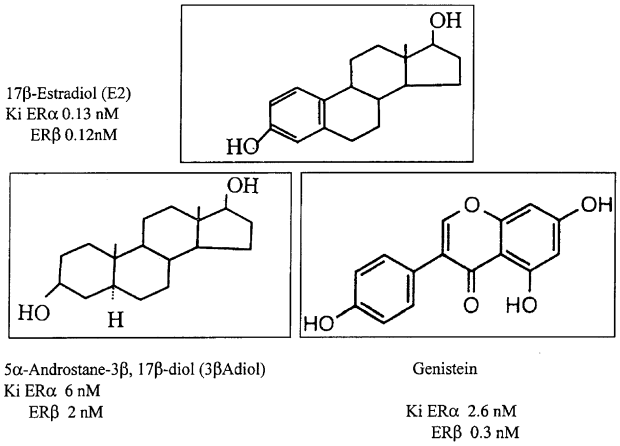

Fig. 2. Chemical structure of the three estrogen receptor ligands discussed in this review. Top: 17β-estradiol, E2; bottom left: genistein; and bottom right: 3βAdiol. The concentrations of each ligand which will displace 50% of bound E2 from ERα and ERβ are listed as K_i.

has not been carefully investigated but indications are that ERβ does not interact with those chaperones that normally interact with ERα in low salt buffer.

In addition to the differences in ligand binding and tissue distribution referred to above, ERα and ERβ also differ in their cofactor interactions and their transcriptional activities. In the mammary gland, the steroid receptor coactivator (SRC-1) is coexpressed with ER in stromal cells but not in epithelial cells [20]. This difference may explain why the response of the stroma to E2 differs from that of the epithelium. Whether or not differences in interaction of ER with coactivators can account for alterations in signaling in normal and disease states is not known but overexpression of the ER coactivators AIB1/SRC-3, AIB3/ASC-2/RAP250 and TRAP220/PBP have been found in breast and ovarian cancer [21].

2. Transcriptional activities of ERα and ERβ

Many but not all of the transcriptional activities of ERs are initiated when the receptor binds to specific DNA sequences in the promoter region of the target genes. The classical consensus ERE is directly bound by ERα and ERβ and at this site ERβ is a weaker transactivator [22–26]. However, there are important DNA binding sites for ER other than ERE where ERβ has different, sometimes even opposite, effects from those of ERα, e.g. at AP-1 and Sp-1 sites. Several well-known gene promoters are regulated by AP-1 sites and in opposite directions by ERα and ERβ [27–30]. On the AP-1 site of the collagenase promoter, E2 elicits transcriptional activation with ERα, but represses it with ERβ [6]. Although the AP-1 site has been used as a model, showing that there are major transcriptional differences between ERα and ERβ, the ER–AP-1 regulation is promoter-specific rather than representing a general mechanism. For example, ERα and ERβ have similar effects on the tumor necrosis factor α promoter even though expression is regulated through an AP-1 site [31].

3. Regulation of expression of ER

A part of the promoter of both the human and mouse ERβ has been analyzed [32,33]. A 2.1-kb sequence of the 5′-flanking region of the human ERβ gene shows that there are regulatory regions including Alu ERE, Oct-1, AP-1 and SP-1 sites. The Alu ERE sequence is thought to be responsible for receptor autoregulation. E2 induces 30–40-fold increased ERβ expression in the T47D human breast cancer cell line. A 600-bp-long mouse ERβ 5′-flanking region revealed the presence of a TATA-like motif and other putative transcription factor binding sites such as GATA-1/2, AML-1/2/3, Nkx-2, CdxA, SRY, E-box, and half-sites of EREs [33]. Of these *cis* elements, GATA-1/2, AML-1/2/3, Nkx-2, CdxA, and SRY are also found in the distal promoter of the ERα gene. The similarities and differences in the ERα and ERβ promoters predict that expression of the two ERs may be coordinately regulated by certain transcription factors and may also have unique cellular and tissue distribution patterns.

ERβ and ERα are regulated in a tissue- and/or cell type-specific manner and E2 itself seems to play an important role in this regulation. E2 can have opposite effects on the expression of ERβ and ERα. In bone mesenchymal stem cells it increases ERα and decreases ERβ, [34] in omental adipose tissue E2 increases ERβ and decreases ERα [35]. The existence of imperfect ERE in both human and mouse ERβ promoters suggests that this response element may be involved in the E2 regulation of ERβ expression. E2 and gonadotropins are the most potent down-regulators of ERβ in several tissues. E2 treatment decreases ERβ expression in the immature rat uterus [36], prenatally and neonatally estrogenized male rat pituitary and in gonadotropin-releasing hormone neurons [37,38]. Gonadotropins down-regulate ERβ in the granulosa cells, probably via a cAMP-mediated pathway [39–41]. However, in the newborn anterior pituitary, in the prostate and in cultured breast cancer cell line T47D, E2 treatment increases ERβ expression [42–44]

4. What knockout mice have taught us about ER action

Several mouse models for the study of estrogen function are available today. These include the knockout of aromatase (Ar−/−), ERα (ERα−/−), ERβ (ERβ−/−), as well as the knockout of both ERα and ERβ (ERα−/−ERβ−/−). Since aromatase (CYP19) is the enzyme which converts C19 steroids to estrogens, its inactivation means that these mice are completely free of E2 and all of its metabolites. Complete characterization of the abnormalities in all these four E2-related gene knockout models is still under extensive investigation but the phenotypes of these mice have provided us with an outline of the roles of E2 and its receptors. It is clear that, from the differences between the three single knockout mouse models, both ERα and ERβ have distinct non-redundant physiological roles [45,46]; in addition, the relatively milder phenotype of Ar−/− mice [47,48] suggests the presence in the body of estrogenic molecules other than and in addition to E2.

5. The uterus

As one of the major classical estrogen target tissues, the uterus of all the three single knockout models demonstrates the essential role of E2 signaling for the normal functions of the uterus. This organ is considered an ERα-predominant tissue, in which ERα is expressed in all cell types [49]. However, it is not free of ERβ. Very soon after its discovery, attempts were made to detect ERβ RNA in rodent uterus with RNase protection assays and Northern blotting. These methods were not sensitive enough, ERβ was not detected and it was concluded that ERβ is not important for the uterus [50]. The importance of ERβ in the uterus has been evaluated in both rodents and primates [51]. In the uterus, the majority of cells are stromal cells. With a more sensitive method for mRNA detection, reverse transcriptase-polymerase chain reaction, we now know that there is a fraction of the uterine stromal cells which harbor high levels of ERβ [36].

The expression of ERβ in the rodent uterus has been further evaluated with two more suitable methods, in situ hybridization and immunohistochemical staining, both of which reveal the cell-specific expression of ERβ [51,52]. These studies showed that most of the cell types in the rat uterus (luminal epithelial cells, glandular epithelial cells, stromal cells, smooth muscle cells and vessel endothelial cells), harbor ERβ mRNA. But it is the subepithelial stromal cells which contain high levels of ERβ. We, and others, have shown that in the mature uterus, ERβ is expressed in the periluminal stromal cells which are destined to undergo decidualization once implantation

occurs. Although decidualization in ERβ−/− mice has not yet been studied, some available data indicate that it is ERβ rather than ERα which mediates this crucial process during implantation [54–56].

Both E2 and ERα are proliferative factors in the uterus. This was confirmed in both Ar−/− and ERα−/− mice whose uteri are hypoplastic. Loss of ERα results in E2-unresponsiveness of some known E2 target genes, e.g. lactoferrin [53]. However, ERα and E2 do not appear to be necessary for the prepubertal development of the uterus. In both Ar−/− and ERα−/−, there is a smaller but morphologically normal uterus, with luminal epithelium, glandular epithelium, endometrium and myometrium. The ERβ−/− mouse uterus is hypersensitive to the proliferative actions of E2. It appears that ERβ has a modulatory role on ERα in the uterus [36]. In addition, the observation that the uterus in ERα−/− mice is bigger than it is in ERα−/−ERβ−/− mice may indicate that in the absence of ERα, ERβ can stimulate the growth of the uterus.

The knockout mice revealed that the progesterone receptor (PR), a long used marker for the transcriptional effects of E2, is not an exclusively ERα-regulated gene. Clearly, regulation of PR in the uterus is not as simple as was thought. Both PRA and PRB are detectable by Western blot analysis in the uterus of ERα−/− mice but in reduced amounts compared to wild-type (wt) mice. The PR in the ERα−/− uterus is fully functional, because progesterone induces its target genes, calcitonin and amphiregulin, and stimulates full decidualization of the uterus [54]. In response to E2, PR is down-regulated in the luminal epithelium and up-regulated in the glandular epithelium and stroma. The down-regulation of PR by E2 in the luminal epithelium is complex and involves participation of both ERα and ERβ.

6. The ovary

It was not until 1997, when Hurst and Leslie transfected ERE chloramphenicol acetyltransferase plasmid into granulosa cells, that it became clear that there were functional ERs in these cells [57]. These studies were done before the existence of ERβ was widely recognized. With ERβ antibodies, the ER status in the ovary has been widely investigated. Generally, ERβ is localized in the granulosa cells of growing follicles and may also be expressed in some stromal cells [58,59]. ERα is mainly expressed in the theca cells and some stromal cells [58,60]. Granulosa cells may not be free of ERα but this remains a debated issue [61].

The ovary is a tissue in which the individual roles of E2, ERα and ERβ are clearly delineated. E2 is mainly produced in the granulosa cells of the ovary during female reproductive age, and ERα is mainly expressed in the interstitial stromal cells and theca cells while ERβ is predominantly expressed in the granulosa cells (Fig. 3B). It seems that neither aromatase, ERα nor ERβ is necessary for embryonic development of the ovary, because the females of all three mouse models have morphologically normal ovaries before puberty. Although the ovaries of both ERα−/− and Ar−/− mice display an age-dependent hemorrhagic cystic phenotype, these abnormalities appear not to be directly related to the loss of ERα or E2 from the ovary itself. The ovarian abnormalities of Ar−/− and ERα−/− mice appear to be due to the increased serum luteinizing hormone levels. When ERα−/− mice receive gonadotropins, they do ovulate and form corpora lutea capable of producing progesterone [62]. These data indicate that ERα is not directly involved in the process of ovulation.

Hemorrhagic cysts appear in Ar−/− mouse ovaries at around 10 weeks of age when animals are kept on a soy-

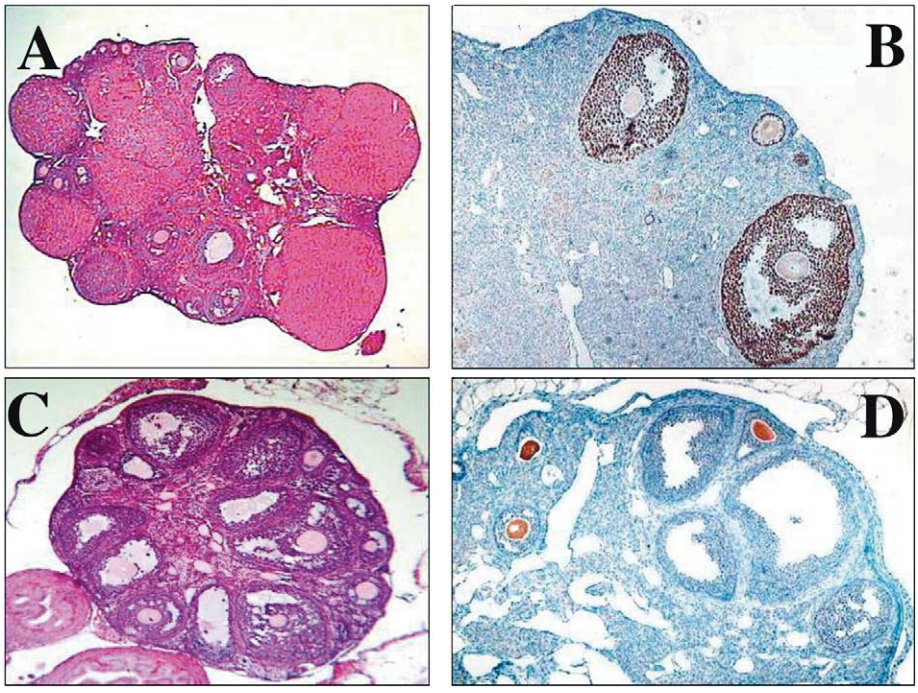

Fig. 3. Comparison of ovaries from wt and ERβ−/− mice. Morphological appearance of wt (A) and ERβ−/− mouse (C) ovaries showing abundant corpora lutea in wt but not in ERβ−/− ovaries. In B the localization of ERβ in granulosa cells of wt mice is illustrated. Fixed sections of ovaries from wt (B) and ERβ−/− mice (D) have been stained for ERβ with a C-terminal antibody.

free diet [47], while ERα−/− mice show the phenotype just at the commencement of sexual maturity around 5 weeks of age [66]. The delayed appearance of the ovarian phenotype in Ar−/− mice indicates that Ar−/− mice may not be estrogen-free, though they are E2-free. Despite this, ovaries of ERα−/−ERβ−/− and Ar−/− mice have a unique phenotype not presented in either ERα−/− or ERβ−/− mice. This is the appearance of Sertoli-like cells in the ovary [64,65]. In addition, in ERα−/−ERβ−/− ovaries there are no preovulatory follicles or corpora lutea and the granulosa cells are stratified. All of this suggests that the presence of both ERα and ERβ is crucial for the normal function of the female gonad.

The ovaries of ERβ−/− mice are distinctly different from those of Ar−/− and ERα−/− mice. Instead of forming hemorrhagic cysts, the ERβ−/− mouse ovary shows accelerated follicular atresia and severely reduced ovulation [46,63]. Corpora lutea are rare (Fig. 3A). Gonadotropin treatment does not appear to increase either the number of oocytes or the number of corpora lutea in ERβ−/− mice. These data suggest that there is a direct involvement of granulosa cell ERβ in the process of ovulation [66]. Though some reports describe the female ERβ−/− mice as subfertile, in our lab we have not been able to generate more than a few pregnant ERβ−/− mice over three years of trying. There is quite a large variability in the degree of subfertility of ERβ−/− mice. The reason for this is not clear. In our colony, ERβ−/− females very rarely become pregnant. Of those who do deliver pups, the litter size is small (one to three pups), and most of the dams eat their pups shortly after birth. We have come to the conclusion that the atretic follicles of the ERβ−/− mouse ovary is only one factor contributing to ERβ−/− mouse subfertility. There appear to be additional uterine and behavioral abnormalities in female ERβ−/− mice.

7. The mammary gland

ERβ is found in both the epithelial cells (ductal and lobular) and stromal cells and even during embryogenesis in the rodent mammary gland [67]. ERα is expressed in both the ductal epithelium and stroma, not in the lobular epithelium. In the epithelium, 60–70% of cells express ERβ at all stages of breast development. Cells co-expressing ERα and ERβ are rare during pregnancy, a proliferative phase, but they represent up to 60% of the epithelial cells during lactation, a post-proliferative phase [13]. One of the most fascinating aspects of ERs in the breast is their dual role in both proliferation and differentiation. It still remains unresolved whether the proliferative effects of E2 on the epithelium are exclusively mediated by growth factors released from the stroma under E2 stimulation. During pregnancy, the proliferating cell nuclear antigen is not expressed in ERα-positive cells and is observed in 3–7% of ERβ-containing cells. More than 90% of ERβ-expressing cells do not proliferate [13].

Severe retardation of mammary gland development has been found in both Ar−/− and ERα−/− mice. In adult females of both genotypes, the mammary glands are rudimentary. Though ERα is expressed in the epithelium of normal mammary gland, and E2 can induce epithelial growth in the presence of progesterone, the mammary gland phenotype in ERα−/− mice appears not to be due to the local absence of ERα in the gland itself. When wt pituitary glands were transplanted into ERα−/− mice, their mammary glands responded to E2 and progesterone in terms of ductal branching and lobular growth [68]. These responses may be due either to the presence of ERβ in the gland, to the effects of the aberrant ERα splice variant observed in ERα−/− mice [69], or to non-receptor-mediated action of E2.

The mammary glands of prepubertal ERβ−/− mice are morphologically indistinguishable from those of wt littermates. It appears that, although ERβ is expressed in the mouse mammary gland, it is not necessary for ductal growth of the gland. However, ultrastructural analysis [70] revealed a role for ERβ in organization and adhesion of epithelial cells. This was particularly evident in lactating glands, where lack of ERβ resulted in larger alveoli and a reduced amount of secretory epithelium, increased interepithelial distance and reduced extracellular matrix and lamina basalis. Levels of the adhesion molecules E-cadherin, connexin 32, occludin and integrin α2 were reduced and no zona occludens was detectable.

8. The ventral prostate

In the ventral prostate ERβ is abundant and ERα absent or very low. Before the discovery of ERβ, ERα had been localized exclusively in the prostatic stroma of several species and thus the effects of E2 in the prostate epithelium were thought to be indirectly mediated by stromal ER [71,72]. In situ hybridization and immunohistochemical staining with different ERβ antibodies have revealed that ERβ is localized in the prostatic epithelium in most species and that direct effects of E2 on the epithelial cells are possible [14,73–75]. Currently our views on the action of E2 in the prostate epithelial cells are being revised to accommodate these new findings.

Hyperplastic prostates have been described in Ar−/− [76] and ERβ−/− mice [14] but not in ERα−/− mice. Because no hormonal alteration has been found in the ERβ−/− mouse serum, epithelial hyperplasia in the ventral prostate appears to be directly due to loss of ERβ in the prostate. However, the hyperplastic phenotype of Ar−/− prostate could, in addition to the loss of ERβ, also be due to the increased androgen and prolactin levels in the Ar−/− mouse serum.

9. The CNS

ERα-containing neurons are located in the medial preoptic area, periventricular nucleus, arcuate nucleus, ventromedial nucleus, and amygdala, all areas involved in gonadally regulated functions [77,78]. ERβ is expressed in the cerebral cortex, hippocampus, as well as paraventricular nucleus and the supraoptic nucleus areas where ERα is not found [79,80]. The two receptors are colocalized in the preoptic area, bed nucleus of the stria terminalis and throughout the lower brainstem. The distribution pattern suggests different functions for the two receptors in the brain. It could be anticipated that brain phenotypes of the mice in which either of these receptors has been inactivated should be different. No morphological changes have been reported in mice lacking ERα but there are striking morphological abnormalities in the brains of ERβ−/− mice [81,82].

In the brains of ERβ−/− mice, layers 2 and 3 of the somatosensory cortex are very reduced and there is a severe neuronal deficit throughout the cortex. In addition, particularly in the substantia nigra, neuronal cell bodies are small and there are large vacuoles surrounding the cell nuclei. In the

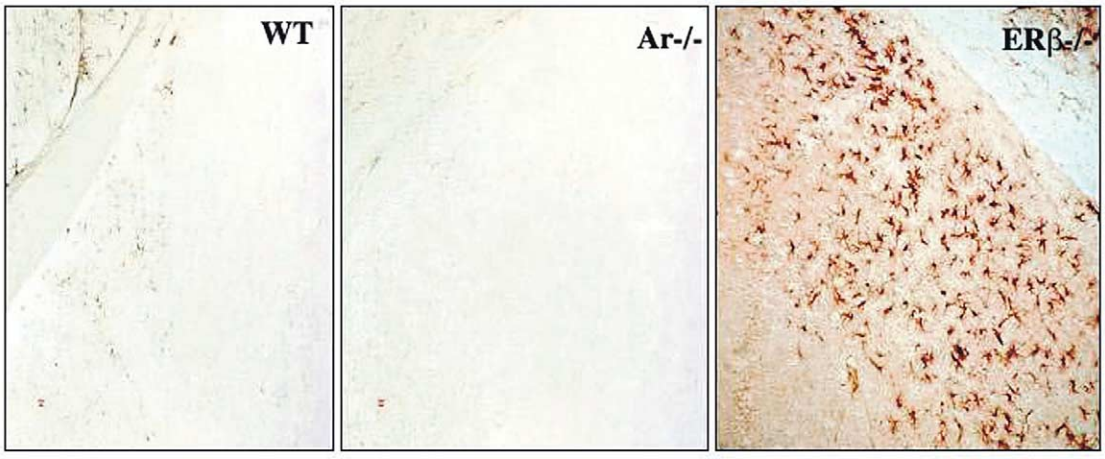

Fig. 4. Astroglia in the medial amygdala nucleus of male wt (left), Ar−/− (middle) and ERβ−/− mice (right). Astroglia are detected by the marker protein GFAP. As shown, there are very few astroglia in the medial amygdala of normal and Ar−/− mouse, but many are seen in the ERβ−/− mice.

limbic system there is a remarkable proliferation of astroglia. Further investigation revealed that there is a problem of neuronal migration in the absence of ERβ [82]. Although proliferation appears to be normal, there are fewer neurons in the cortex and abnormal migration patterns are evident. Even though abnormalities in the brains of Ar−/− mice have been reported [83], unlike ERβ−/− mice, there is no evidence for proliferation of astroglia in Ar−/− mice (Fig. 4). The differences between the brains of ERβ−/− and Ar−/− mice suggest that Ar−/− mice are not completely estrogen-free and hint at an alternative estrogen or an alternative route for activation of ERs in the body.

10. Alternative estrogen receptor ligands in the body

Two of the major metabolites of the potent androgen 5α-dihydrotestosterone (DHT) are also potent hormones. These two steroids, 5α-androstane-3α,17β-diol (3αAdiol) and 5α-androstane-3β,17β-diol (3βAdiol; Fig. 2), although they are epimers, have very different biological actions. 3αAdiol is androgenic, and 3βAdiol is estrogenic. 3αAdiol functions as a weak androgen by itself or, through the action of 3α-hydroxysteroid dehydrogenase (3αHSD), is reconverted to the potent androgen DHT [84]. It has no estrogenic effects. 3βAdiol is formed from DHT through the action of 3βHSD. It does not itself have androgenic activity [85] but it can be reconverted to DHT since 3βHSD activity is reversible. The estrogenic activity of 3βAdiol has been well documented. It can displace E2 from ERα [86,87]. Compared to E2 its affinity is lower by 10-fold for ERβ and 30-fold for ERα [88].

In some in vivo experiments, it has been difficult to show estrogenic activity of 3βAdiol. Uterine growth and vaginal stratification were observed when high doses of 3βAdiol (14 mg/kg body weight) were used to treat hypophysectomized rats [89]. With a lower dose (1 mg/kg body weight) there were no uterotrophic effects but 3βAdiol delayed the onset of first ovulation [90]. Furthermore, in vivo, 3βAdiol has no effect on serum gonadotropin levels while 3αAdiol effectively suppresses gonadotropin serum concentration. These in vivo data have led some endocrinologists to believe that 3βAdiol has no important physiological functions itself, and is only as a metabolite of androgen.

The physiological levels of 3βAdiol make its role intriguing. In the prepubertal female rat, the serum level of 3βAdiol is quite high, around 100 ng/ml. After the first ovulation, it decreases to 10 pg/ml [91]. The origin of 3βAdiol in the immature female is the ovary. Not much attention has been paid to the steroids secreted from the immature ovary because it does not synthesize estradiol. However, the immature ovary is not a silent tissue. Even though their physiological significance is not understood, substantial quantities of 3αAdiol and 3βAdiol are synthesized in the immature ovary [91]. In the immature testis, as in the immature ovary, a large amount of 3βAdiol is produced, with a peak between postnatal day 10 and day 25. Thereafter, plasma levels decline [92]. At maturity, the plasma concentration of 3βAdiol in the male rat is around 200 pg/ml [93]. This is about 20 times higher than the level of estradiol in the adult male rat [94]. Considering that its affinity for ERβ is 10-fold lower than that of E2, 3βAdiol could well serve as a physiological ligand for ERβ.

Tissue levels of 3βAdiol are regulated by the enzyme 3βAdiol hydroxylase, a member of the cytochrome P450 superfamily, identified as CYP 7B1. 3βAdiol is hydroxylated at the 6α or 7α positions and these triols are the final, inactivated urinary metabolites of DHT. 3βAdiol hydroxylase activity is high in the prostate, brain, and pituitary but it is not detectable in the liver [95,96]. It seems that the estrogenicity of 3βAdiol in a cell is determined by the presence or absence of CYP 7B1 [97]. In cells where CYP 7B1 is highly expressed 3βAdiol will be inactivated and will not be estrogenic. This might explain why low doses of 3βAdiol do not regulate gonadotropin secretion from the pituitary.

11. ERβ splice variants

There is one further confounding factor in the estrogen action equation. This factor is the splice variants of ERβ. Several splice variants of ERβ have been described (Fig. 1C; see [98] for review). One which is of particular interest because of its expression in human cancers is ERβcx [6]. In this splice variant, an alternative exon 8 is utilized. The last 61 C-terminal amino acids (exon 8) are replaced by 26 unique amino acid residues. Due to the exchange of the last exon, ERβcx lacks amino acid residues important for ligand binding

and those that constitute the core of the AF2 domain. It therefore does not bind E2 and has no capacity to activate transcription of an E2-sensitive reporter gene. ERβcx shows preferential heterodimerization with ERα rather than with ERβ, inhibiting ERα DNA binding. Functionally, the heterodimerization of ERβcx with ERα has a dominant negative effect on ligand-dependent ERα reporter gene transactivation. Emerging studies on the ERβ isoforms in prostate and breast cancer indicate that ERβcx is expressed as a protein [99] and its presence in cancers can influence prognosis and recurrence.

12. Concluding remarks

The known or suspected functions of many genes have been confirmed and new unsuspected functions of many genes have been revealed with knockout mice. While clear roles of ERα and ERβ are being intensively studied in experimental models, aberrant ER expression in several human diseases is also being examined and studies suggest important roles of both receptors in malignancies of the breast, prostate, lung, colon and ovary and in non-malignant diseases such as polycystic ovarian syndrome, endometriosis, cardiovascular disease, obesity, osteoporosis and degenerative diseases of the CNS. Because of the existence of ERβ isoforms which can have quite different biological functions, information about E2 action in human diseases must be considered incomplete if ERβ and its isoforms as well as ERα are not specified.

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