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Update on animal models developed for analyses of estrogen receptor biological activity☆

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Abstract

Targeted disruption of the different ER genes has generated experimental animal models that are very useful in evaluating the distinct and cooperative roles of the two estrogen receptors, ERα and ERβ, in reproductive but also non-reproductive tissues of both sexes. Phenotypic analysis has provided definitive experimental findings for estrogen receptor mediated physiological actions, involving ERα in uterine, mammary gland and neuroendocrine sites. ERβ is involved most dramatically in the ovary as is ERα. More detailed studies in combination with tissue specific or inducible ER knock outs will be important for future research.

Keywords: Estrogen receptor; Knock-out mice; Phenotypes; Reproductive tract

1. Introduction

Estrogens biological effects were first identified some years ago related to reproduction and fertility. Since that time, physicians and researchers have identified a broad spectrum of organ systems that respond, or have been suggested to respond to estrogen hormones, besides the female reproductive tract and mammary gland; these include the skeleton, cardiovascular system, immune system, and central nervous system. Following the work of the Jensen and Gorski laboratories in the early 1960s, it is now clear that estrogens exert most of their effects through specific estrogen receptor proteins. Two different estrogen receptor proteins, which function as ligand activated transcription factors; estrogen receptor α (ERα) [1,2]; and estrogen receptor β (ERβ) [3–5], have been cloned and characterized. As a means to study each of these estrogen receptors and provide an understanding of their biological roles, mice were generated lacking either a functional ERα or ERβ, referred to as αERKO and βERKO mice, respectively [6,7]. More recently, mice lacking both estrogen receptors, αβERKO mice, also have been generated [8,9]. These mice are viable and live a full life span. This was somewhat surprising since based on the prevailing view that estrogens were essential for prenatal survival, and would have implicated the possibility of another estrogen receptor besides ERα and β for mediating the effects. Subsequent generation of viable Aromatase knock out mice (ArKO) also refuted the essential requirement for estrogen during development [10]. Studies in rodents showed that the tissue distribution of the two receptors differs: ERα is expressed in many different tissues, including the female and male reproductive tract, skeletal and cardiac muscle, kidney, liver, hypothalamus and pituitary gland. ERβ expression is more limited and is expressed in noticeably high amounts in the ovary, male reproductive tract, sperm, lung and areas of the hypothalamus. αERKO mice express ERβ at comparable wild-type levels and vice versa βERKO mice express ERα at normal levels indicating that the two receptors do not influence the expression of each other. αERKO and βERKO mice turned out to be highly useful in understanding the distinct roles of both estrogen receptors in various tissues. These knockout models can be suitable for evaluating individual receptor-mediated actions of particular ligands or selective estrogen receptor modulators (SERMS) in vivo because disruption of a specific receptor type generates mice that will show responsiveness to the other estrogen receptor. We describe in this report several of the phenotypes seen in specific organ systems of the different estrogen receptor knockout mice. A more detailed

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description has also been reviewed ([11] and references therein).

2. Female reproductive phenotype

2.1. Uterine phenotypes

Estrogens and progesterone, as well as many growth factors such as IGF-1 and/or EGF/TGFα, play key roles in female reproductive tract function. Under the influence of these steroids, the uterus is prepared for a possible establishment and maintenance of pregnancy. Estrogen stimulates proliferation of the uterine epithelum, and induces the progesterone receptor (PR). Progestins, subsequently, induce stromal cell proliferation and differentiation and oppose the estrogen-induced epithelial cell proliferation. ERα appears to be the predominant estrogen receptor in the adult mouse uterus [12]. ERβ is detectable in the uterus of both wild-type and αERKO mouse uteri, but only at very low levels. Both αERKO and βERKO mice have normally developed uteri but differ in their morphology [6,7]. Uteri of adult βERKO mice show no appreciable morphology or histological differences to that of wild-type mice and demonstrate a full response to circulating ovarian hormones [7,9]. In contrast, uteri of adult αERKO female mice are hypoplastic and have lost their uterotropic estrogen-responsiveness, as measured by tissue bioassays [6,13]. Wild-type mice respond to the three different types of agonists (estradiol, tamoxifen or diethylstilbestrol) in a similar way, showing an increase in uterine wet weight. αERKO mice, however, show no uterine stimulation after administration of any of these three agonists. In addition, not only is the uterine growth and morphogenesis lacking in αERKO mice, but also histological effects of hyperemia (vascular swelling) and water inhibition, classical early estrogen responses, which link these responses to a functional ERα. Further analysis revealed that estrogen-treated αERKO uteri did not demonstrate increased DNA synthesis nor increased gene expression of PR, lactoferrin and glucose-6-phosphate dehydrogenase (G6PD), all known estrogen-induced genes in the adult uterus, [14]. Basal levels of PR mRNA and protein in αERKO uteri appear not to differ from those in wild-type. Additionally, PR-mediated progesterone actions appeared to be preserved in αERKO uteri as evidenced by gene regulation of calcitonin expression and amphiregulin [15].

The uterine phenotype observed in the αβERKO is quite similar to the αERKO [8]. However, Dupont et al. [9] reported an alteration of αERKO uterine phenotype in the αβERKO uterus. The uterine diameter and wall thickness was reduced in the αβERKO uterus in comparison with the αERKO uterus. They suggested at least some compensatory role may exist for ERβ activity in the mouse uterus. In contrast, other investigators have proposed that ERβ functions to attenuate the activity of estrogen through ERα.

2.2. The ovary

Folliculogenesis and steroidogenesis are two basic endocrine and physiological actions occurring in the ovary. Understanding whether a role exists for estrogen receptor in these ovarian functions has been very difficult. This is due to the fact that the action of estrogen had to be studied in the tissue that is making the hormone itself. Interference with ERα or ERβ action through targeted gene disruption in mice provided us with appropriate genetic models for evaluating the possible role of estrogen and ER mediated actions in the ovary. Some of the interesting phenotypes that have evolved from the ER knockout mice confirm the importance of ER signaling and activity in the ovary.

In contrast to the uterus, in which ERα can be considered the predominant receptor, both ERα and ERβ are clearly present in the adult rodent ovaries [3,12,16]. The receptor distribution pattern, however, differs among the different ovarian cell types. ERβ is predominantly localized in the granulosa cells of the ovary, whereas ERα expression is limited to the thecal and interstital cells. Analysis of αERKO and βERKO females revealed distinct ovarian phenotypes consistent with the expression patterns [6,7,17]. It appears clear that ERα and ERβ expression and signaling is not essential for development of the ovary. αERKO females were found to be infertile in continuous mating studies, whereas βERKO females are subfertile. Histological analysis of the αERKO ovary showed a polycystic or PCOS type morphology, with enlarged hemorrhagic, cystic follicles, no corpora lutea and no indication of ovulation. Further analysis revealed that the anovulation develops and worsens as the αERKO females are aging [17,18]. Further support for a PCOS type condition comes from findings that αERKO females show increased obesity and insulin resistance similar to the clinical condition [19] which is also seen in ArKO mice [20]. Immature αERKO females studied prior to development of the overt ovarian phenotype are responsive to superovulation treatment, but with a reduced response when compared with immature wild-type females.

Disruption of the ERα gene severely affects the negative feedback action of estradiol on the hypothalamic-pituitary axis, resulting in highly elevated androgen, estradiol and LH serum levels. The chronic elevation of serum LH levels is considered the major cause of the ovarian phenotype seen in αERKO females. Moreover, the same polycystic ovarian phenotype is seen in transgenic females overexpressing LH [21]. This hypothesis is supported by the finding that in GnRH-antagonist treated αERKO females, serum LH levels are suppressed to wild-type range and the polycystic ovarian phenotype is prevented [22], consistent with some of the current clinical treatments for PCOS.

Ovaries from βERKO females, in contrast, have a totally different phenotype [7]. In these ovaries, follicles are seen at various stages of development, from primordial up to large antral follicles. Superovulation treatment results in βERKO females with an obvious ovarian phenotype,

exhibiting numerous ovulatory but unruptured (trapped) follicles. Serum LH, FSH and estradiol levels are within the normal wild-type range. The observed subfertility in βERKO females appears to be primarily caused by this compromised ovulatory efficiency, as there are no indications that the uterus was dysfunctional. Whether this dysfunction is intrinsic to the βERKO ovary or caused by a disturbed hypothalamic-pituitary axis is currently being determined. The ovarian phenotype found in mice lacking a functional progesterone receptor [23] or prostaglandin synthase-2 (PGS2) [24] is very similar to that observed in βERKO females. Therefore, expression of these genes is currently being evaluated in βERKO ovaries in addition to ERα and Cyclin D2 expression.

The α/βERKO adult ovaries demonstrate a phenotype that is quite distinct from that seen in either αERKO or βERKO ovaries [8,9] indicating that both receptor types are required in the ovary. α/βERKO ovaries contain follicles that predominantly reach the small antral stage, with only a few follicles possessing a large antrum and no corpora lutea. Surprisingly, structures observed within these ovaries resembled testicular cord-like structures containing Sertoli-like cells but were not ovotestes since they contained no evidence of spermatogenesis. These structures are only observed in the adult α/βERKO ovaries and not in the prepubertal ovaries. The granulosa cells of these "sex-reversed" follicles have undergone redifferentiation to a Sertoli cell phenotype, determined by both morphological and biochemical markers. Thus, ERα and ERβ actions appear not to be essential in ovary determination but both receptors are involved in maintaining the proper differentiation state of the granulosa cells.

Mice lacking the ability to produce estrogen due to targeted disruption of the aromatase gene, ArKO mice, have an ovarian phenotype which, from initial reports, appears more similar to that seen in αERKO females [10]. Serum levels of LH, FSH and testosterone are elevated in ArKO females, but there is no detectable estradiol. In contrast to αERKO females, which have normal levels of serum FSH but elevated levels of estradiol and LH. ArKO ovaries contain follicles of different developmental stages but no corpora lutea and are infertile. With age, the ArKO ovaries develop into polycystic hemorrhagic ovaries, similar to what is seen in the αERKO ovaries [25]. Obviously, the lack of both ERα and ERβ signaling appears to lead to a different ovarian phenotype than lack of estrogen alone. Although this difference cannot be explained at the present time, different hypothesis can be proposed.

Polypeptide growth factors, including epidermal growth factor and insulin-like growth factor I, could stimulate ER activity in an estrogen-independent manner, although the significance in vivo related to normal physiology is yet to be determined [26,27]. Due to the presence of both a functional ERα and ERβ, such non-estradiol estrogen receptor signaling pathways will still be functional in ArKO females. A second possible explanation for the difference between ArKO and αERKO mice is exposure to maternal estrogens of the developing ovary. Although ArKO females have undetectable serum estrogen levels, these animals are still responsive to estrogens. During prenatal development, maternal estrogens can induce early developmental changes in the ovary of ArKO female fetuses. This might lead to the observed and different ovarian phenotype in ArKO and αERKO females at a later stage of ovarian differentiation. Recent reports of ArKO mice exposed to a soy-free diet, eliminating phytoestrogens, has shown more dramatic phenotypes [28].

2.3. Phenotypes of the mammary glands

Besides the uterus, the mammary gland is an organ system that is highly responsive to estrogen and development of hormonal therapeutics involving SERM activities. The mammary gland consists of a ductal and lobuloalveolar network, embedded in stromal tissue [29]. Early experimental and clinical findings show that at birth, a rudimentary ductal system is present in the nipple area. The ductal system expands under normal influence during puberty through proliferation at the terminal end buds of each branch. Pregnancy and lactation result in changes in which the ductal system undergoes further branching and formation of lobuloalveolar structures. Many studies have implicated a role for both estrogen and progesterone in mammary gland differentiation and function. Estrogen stimulates proliferation of the mammary epithelial cells, and induces the progesterone receptor protein. Progesterone, in turn, induces formation of lobuloalveolar structures. Analysis of the mammary glands of both ER and PR knockout mice supported these previous observations [23,30]. Additionally, these mouse models have proven to be very useful in studying the distinct roles and effects of these two hormones in mammary gland function.

Levels of ERα mRNA are highly expressed in the adult mouse mammary gland. ERβ is undetectable by using a RNAse protection assay but is detectable by RT-PCR in adult mouse mammary glands [11]. Mammary glands of βERKO females appear to be comparably developed to those of wild type mice. βERKO females are able to lactate and nurse their young following pregnancy. In contrast, αERKO mammary gland shows normal prenatal and prepubertal development but remains rudimentary after puberty, lacking epithelial branching and lobuloalveolar development when wild glands will grow and expand [30]. The α/βERKO mammary gland phenotype resembles that of αERKO adult females. Tissue recombinant experiments have shown that presence of ERα in the stromal compartment is essential for ductal growth and branching [31].

PR gene stimulation is a well-known estrogen-response in the uterus but also in the mammary gland. In PR knockout mice, ductal development does occur in the mammary gland but lobuloalveolar development is lacking [23]. In αERKO mammary glands, basal PR expression is significantly reduced and estrogen stimulated increase in PR gene expression is lost [30]. The lack of PR induction may contribute to the αERKO mammary gland phenotype. In addition,

disruption of the ERα gene also affects the positive feedback action of estradiol on the prolactin (PRL) secreting cells in the pituitary. PRL is essential for full mammary gland development as shown by the presence of abnormal mammary glands in PRL receptor knockout mice [32]. PRL mRNA was dramatically reduced in the αERKO pituitary and serum PRL levels are lowered in the αERKO [33]. Pituitary transplantation studies were used to determine whether the observed mammary gland phenotype in αERKO females was due to the low PRL levels secreted by the pituitary [34]. An elevation of PRL and mammary gland development could be achieved in αERKO female mice transplanted with normal pituitaries. Mammary gland development, however, was only seen in transplanted animals with intact ovaries and not in ovariectomized recipients, implicating the need for additional ovarian factors. Treatment of αERKO female animals with high dose estradiol and progesterone induced alveolar development and branching in the mammary gland, whereas progesterone alone or in combination with PRL did not. Estradiol alone can cause some growth in the αERKO mammary gland, but the combined action of estradiol and progesterone are needed for the fullest response. It would appear from the studies so far that the phenotype seen in the αERKO female mammary gland results due to a direct action of estrogens on the gland itself but also via indirect mechanisms involving the hypothalamic-pituitary axis.

3. Male reproductive phenotypes

Male fertility and reproduction has been principally thought to be controlled by androgen hormones and their receptor. The generation of αERKO mice, however, indicated that estrogen is also essential for male fertility. Therefore, estrogen based therapeutics may also be effective for treating male fertility.

Male αERKO mice are infertile with extensive dysmorphogenesis and swelling of the seminiferous tubules and disruption of spermatogenesis, reflected by a lower sperm count and decreased sperm motility in comparison with wild-type male mice [35]. Although the presence of both ERβ mRNA and protein has been demonstrated in the male reproductive tract, βERKO male mice are fertile and suffer no apparent reproductive alterations or obvious morphological phenotypes [7,36]. The α/βERKO male mice are infertile. They show an overt and definitive phenotype with respect to dysmorphogenesis of the seminiferous tubules and impaired spermatogenesis. Similarity of this phenotype to that of the αERKO male and absence of any effect in βERKO males suggests that estrogen action in the male tract related to fertility appears to be mainly involving ERα [11,12].

More in depth studies showed that the impaired spermatogenesis is not caused by any defects in the germ cells but is indirectly through disruption in the somatic cells of αERKO male reproductive tract [37]. A role for estrogens in the luminal fluid balance in the head of the epididymis was suggested based on the observation of dilated efferent ducts and a morphologically abnormal epithelium that has lost its ability to reabsorb fluid from the tubules in the αERKO male [35,38]. This leads to the accumulation of fluid in the efferent tubules and testis, eventually producing testis atrophy. This αERKO phenotype can be at least partially reproduced in wild-type animals through blockage of ER action with the pure antiestrogen ICI 182,780 [39]. Although no alteration in hormone androgen action has been detected, serum testosterone levels are slightly elevated in the αERKO male in comparison with those of wild-type males [35]. Findings from the αERKO suggest that there is a definite physiological requirement for estrogen in male reproductive tissues.

It was proposed that the phenotype of male ArKO mice, being estrogen deficient, might be similar to that of the male α/βERKO mice. ArKO male mice, however, appeared much less severely affected compared to male mice lacking ERα alone or both ERα and ERβ. Initial reports showed ArKO male mice are fertile with no morphological changes observed in the testis [10]. Again, this difference might be the result of ligand-independent estrogen receptor activation pathways that are still functional in ArKO male mice and not in the αERKO or α/βERKO male [11].

4. Conclusion

Targeted disruption of the different ER genes has resulted in animal models that are very useful in evaluating the distinct and cooperative roles of ERα and ERβ in reproductive but also non-reproductive tissues. Analyses of the phenotypes has provided definitive confirmatory experimental findings for estrogen receptor mediated physiological actions, but has also uncovered some surprising effects, and in some instances, lack of effects regarding ER activity. More detailed studies in combination with tissue specific or inducible ER knock-outs will be important for future research.

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