Estrogen Receptor Knockout Mice as a Model for Endocrine Research

Vickie R. Walker and Kenneth S. Korach

Abstract

The biological effects of estrogen in mammalian target tissues are important for multiple organ systems including the male and female reproductive tract and the neuroendocrine, skeletal, and cardiovascular systems. Numerous physiological effects of estradiol are modulated by the estrogen receptor (ER), a Class I member of the nuclear receptor superfamily. However, more recent studies have also implicated nongenomic effects of estrogen, which may involve a membrane-binding site. The two forms of the ER are the classical estrogen receptor-alpha (ERα) and the more recently discovered estrogen receptor-beta (ERβ). Gene-targeting techniques were used to generate mice lacking either functional ERα (αERKO), ERβ (βERKO), or both ERs (αβERKO) to provide a model for evaluating estrogen receptor action. These knockout models provide a unique tool to study the effects of estrogen in the context of the whole animal and to discern the role of each ER in various tissues. The reproductive phenotypes as well as some of the nonreproductive phenotypes of the different ERKO models are summarized.

Key Words: estrogen receptor; knock-out mice; reproductive phenotypes; transgenic mice

Estrogen Receptor Structure and Mechanism of Action

Two estrogen receptors (ERs1), alpha and beta, have been identified. Both ERα and ERβ produce a protein product from a separate gene located on different chromosomes, and as Class I members of the nuclear hormone receptor superfamily, are composed of the following six functional domains, labeled A-F: (1) the N-terminal A/B domain; (2) the C domain containing the DNA-binding domain (DBD1); (3) the D domain possessing signals for nuclear localization; (4) the E domain, or the ligand binding domain (LBD1); (5) the C-terminal F domain, which is unique to the ERs among the nuclear receptors and not well conserved; and (6) the N-terminal domain of the ER, which contains a ligand-independent activation function (AF1)-1 (Nilsson et al. 2001) that appears to be more important in ERα than ERβ. The DBD contains two zinc finger structures that play an important role in binding the receptors to specific DNA sequences known as estrogen response elements (EREs) within the regulatory sequences of target genes. The DBD for ERα and ERβ share a high degree of homology (97%) and are therefore likely to bind to the same EREs (Swope and Korach 2003). The E domain mediates ligand binding and receptor dimerization, and establishes ligand specificity and transactivation of target gene expression via the AF-2 domain (Nilsson et al. 2001). The AF-2 domain is critical to ligand-dependent transactivational activity as well as recruitment of coregulator proteins. ERα and ERβ share a moderate degree of homology (60%) in LBD, show similar binding affinities for estradiol, and exhibit comparable binding affinities for a number of natural and synthetic ligands (Couse and Korach 1999a; Nilsson et al. 2001; Swope and Korach 2003).

The classical ligand-dependent mechanism by which ER activates transcription of target genes requires that upon binding to ligand, the LBD undergoes a conformational change that allows interaction with coactivators (Bocchinoso et al. 2000; Moggs and Orphanides 2001; Swope and Korach 2003). The ligand-bound ER undergoes dimerization, binds to ERE sequence in target genes, and induces transcription. In addition to the classical mechanism, ER action can involve ligand-independent activation, in which ER activation results in the absence of estrogen. Ligand-independent activation of ER can be modulated by a number of signaling pathways including growth factors, protein kinase A, and protein kinase C. Estrogen target genes may also be regulated by ER in the absence of EREs. These other mechanisms for ER activation may correspond to other ways of mediating or enhancing the transcription via EREs in situations of low hormone levels (Swope and Korach 2003).

The tissue distribution of the two receptors both overlaps and differs. ERα mRNA is predominantly expressed in the uterus, mammary gland, testis, pituitary, liver, kidney,
heart, and skeletal muscles, whereas ERβ mRNA is expressed in the ovary and prostate. The epididymis, thyroid, adrenals, gonad, and various regions of the brain show relatively equal levels of ERα and ERβ mRNA. Within the tissues that coexpress both receptors, the cellular distribution between the estrogen receptor subtypes differs.

**Generation of ERKO Mice**

**Utility of ERKO Models**

In studying knockout mice that use homologous recombination in embryonic stem cells, scientists have been able to analyze the effects of the absence of a certain gene product on the viability, development, and physiology of the animal. This experimental approach has been used to generate three different knockout models that lack either ERα (αERKO) (Lubahn et al. 1993) or ERβ (βERKO) (Krege et al. 1998) individually, or that lack both ERα and ERβ genes (αβERKO) (Couse et al. 2000; Dupont et al. 2000; Swope and Korach 2003). In Table 1, many of the reported phenotypes observed in mice with a disrupted estrogen receptor are summarized. The ERKO models provide a unique and valuable tool to assess the physiological consequences of the complete lack of ER activity and the role(s) each receptor may play in various tissues.

Expected Mendelian distribution of ER genotypes is seen in all breeding colonies with no bias in sex ratio. Moreover, in all three knockout models, the mice develop normally and have a life span comparable to their wild-type littermates. However, adult mice of each ERKO model exhibit several abnormal phenotypes unique to that model.

**Fertility and Behavior**

Both sexes of αERKO mice are infertile (Couse and Korach 1999a,b; Lubahn et al. 1993). No vaginal plugs or pregna-

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**Table 1 Phenotypes of estrogen receptor knockout mouse (ERKO) models**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>αERKO</th>
<th>βERKO</th>
<th>αβERKO</th>
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</thead>
<tbody>
<tr>
<td><strong>Fertility</strong></td>
<td></td>
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<tr>
<td>Both sexes are infertile</td>
<td>Males are fertile</td>
<td>Subfertile females; infrequent pregnancies and reduced litter size</td>
<td>Both sexes are infertile</td>
</tr>
<tr>
<td><strong>Female Reproductive System</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Uterus</td>
<td>Hypoplastic uterus; insensitive to estradiol; no implantation</td>
<td>Normal response to estradiol; supports normal pregnancy</td>
<td>Resembles αERKO phenotype; insensitive to estradiol</td>
</tr>
<tr>
<td>Ovary</td>
<td>No ovulation; immature follicles; hemorrhagic cysts developing at puberty due to chronic elevated LH; elevated levels of estrogen and testosterone</td>
<td>Normal appearance; reduced ovulation</td>
<td>Granulosa cells undergo transdifferentiation into Sertoli-like cells</td>
</tr>
<tr>
<td>Mammary Gland</td>
<td>Immature; only a ductal rudiment present</td>
<td>Normal structure; normal lactation</td>
<td>Immature; resembles αERKO phenotype</td>
</tr>
<tr>
<td><strong>Male Reproductive System</strong></td>
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<td></td>
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<tr>
<td>Testes</td>
<td>Normal development; testes weight decreases with age; fluid retention and dilation of seminiferous tubules; sperm have poor motility</td>
<td>Normal sexual behavior</td>
<td>Resembles αERKO phenotype</td>
</tr>
<tr>
<td>Mating Behavior</td>
<td>Decreased aggression; disrupted mating behavior</td>
<td>Normal sexual behavior</td>
<td>Males do not mount; disrupted mating behavior</td>
</tr>
<tr>
<td><strong>Nonreproductive Phenotypes</strong></td>
<td></td>
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</tr>
<tr>
<td>Bone</td>
<td>Both sexes are shorter than wild-type; females have decreased bone diameter; males have decreased density</td>
<td>Females have increased density; normal in males</td>
<td>Both sexes are shorter than wild-type</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Estrogen protection retained in vascular injury study</td>
<td>Estrogen protection retained in vascular injury study</td>
<td>Estrogen protection lost in vascular injury study</td>
</tr>
</tbody>
</table>

* LH, luteinizing hormone.
cies were found in matings with αERKO female mice. βERKO males show normal fertility when compared with wild-type males, whereas βERKO females exhibit reduced fertility (Krege et al. 1998). In continuous matings, βERKO females had fewer litters than the wild-type females, and the number of pups per litter was significantly lower. αβERKO males and females are infertile (Couse and Korach 1999a,b; Dupont et al. 2000).

Steroids like estrogens and progesterone are known to be involved in reproductive behavior. The availability of αERKO, βERKO, and αβERKO animals provide unique models to study the precise roles of ERα and ERβ in reproductive behavior. Although βERKO female mice have fewer litters and a reduced number of pups per litter compared to wild-type mice, they do not demonstrate any difference in behavior compared with wild-type animals (Ogawa et al. 1999). In contrast, sexual behavior of αERKO animals is altered. αERKO females did not show the lordosis response and were also deficient in sexual behavioral interactions preceding the lordosis response (Ogawa et al. 1998; Rissman et al. 1997). In addition, αERKO females were often attacked by the male, which may be partially due to the elevated levels of testosterone in αERKO females. The lack of ERα and ERβ in the hypothalamus during development has little effect on male sexual behavior in terms of mounting and sexual attraction toward wild-type females (Couse et al. 2001; Ogawa et al. 1997; Wersinger et al. 1997). αERKO males have slightly reduced levels of intromissions, rarely ejaculate, and have greatly reduced aggressive behavior.

αERKO males are completely insensitive to the activating effects of exogenous estradiol or androgen treatment (Couse et al. 2001; Ogawa et al. 1997, 1998). βERKO males show no abnormalities in sexual behavior. In contrast, αβERKO males exhibit a complete disruption of sexual behavior, from both consummatory and motivational aspects. This characteristic suggests that ERα might be predominantly involved in the ability of the males to behave normally, whereas ERβ-dependent actions are important in the regulation of sexual behavior.

Animal Husbandry and Breeding

No special animal husbandry is necessary to maintain breeding colonies of either of the ERKO mouse lines, and all animals are genotyped by polymerase chain reaction on DNA extracted from tail biopsy. Due to the infertility of both the male and female αERKO, αERKO mice are obtained by breeding heterozygous males with heterozygous females. βERKO mice are obtained by breeding βERKO males with heterozygous females because some of the βERKO females are subfertile. To generate mice homozygous for ERα and ERβ (αβERKO) mice heterozygous for both ERα and ERβ are crossed.

Phenotypes of the Female Reproductive Tract

Uterus

ERα is the predominant ER in the uterus. Adult αERKO, βERKO, and αβERKO females possess all three definitive uterine compartments: the myometrium, the endometrial stroma, and the epithelium. The βERKO uterus appears normal and responds properly to hormones (Dupont et al. 2000; Krege et al. 1998). However, in the αERKO and αβERKO female mice, each compartment is hypoplastic, which results in uterine weights that are approximately one

Figure 1 Phenotypes in the reproductive tissues of the adult female estrogen receptor knockout models. ERKO, estrogen receptor knockout mouse.
half the expected size relative to wild-type littermates (Figure 1). Although the αERKO females are fertile and can support pregnancy, the αERKO and αβERKO females are infertile. Uteri from both αERKO and αβERKO females have lost estrogen responsiveness and exhibit no increase in uterine wet weight after the administration of estradiol. Moreover, no increased expression of the estrogen-responsive genes progesterone receptor (PR\(^R\)), lactoferrin, and glucose-phosphatase gene could be demonstrated in estrogen-treated αERKO uteri (Couse et al. 1995). Nevertheless, PR protein is present in the αERKO uterus, albeit at reduced levels, and PR-mediated actions have appeared to be preserved in αERKO uteri (Curtis et al. 1999). In wild-type mice, estrogen treatment induces PR mRNA and protein in the stroma, and down-regulates PR expression in the uterine epithelium (Emmen and Korach 2003). In contrast, the αERKO females reveal up-regulation, but not down-regulation, of PR in the uterine stroma, suggesting that the role of ER\(\alpha\) in the regulation of PR in the uterus is tissue compartment specific.

Ovary

Both ER\(\alpha\) and ER\(\beta\) are present in the ovary, but the distribution pattern differs among the different ovarian cell types (Kuiper et al. 1996; Sar and Welsch 1999; Schomberg et al. 1999). ER\(\alpha\) mRNA and protein, which are expressed minimally in the ovary, are located predominantly in the thecal layer. ER\(\beta\) mRNA and protein are highly expressed in the granulosa cells of the developing follicles. The lack of ERs appears to have no gross effect on ovarian differentiation. αERKO, βERKO, and αβERKO females all possess normal ovarian morphology at birth and during prenatal development (Couse and Korach 1999a; Krege et al. 1998; Schomberg et al. 1999). In contrast, ovaries of adult knockout mice show distinct phenotypes unique to the loss of ER\(\alpha\), ER\(\beta\), or both (Figure 1).

Adult αERKO ovaries contain hemorrhagic, cystic follicles with no signs of ovulation (Lubahn et al. 1993). Follicles of αERKO females progress through the primordial, primary, and antral stages but do not reach the preovulatory stage.

αERKO ovaries produce and secrete increased amounts of estradiol and testosterone compared with wild-type animals (Couse et al. 1995, 2003). Moreover, the adult αERKO ovary shows increased levels of follicle-stimulating hormone (FSH\(^H\)), FSH receptor, and luteinizing hormone (LH\(^H\)) receptor mRNA compared with wild-type (Emmen and Korach 2003). Superovulation treatment of immature females before development of the cystic ovarian phenotype showed that αERKO females can ovulate, although the number of oocytes derived from these knockout females was significantly less than immature wild-type females (Couse and Korach 1999a,b). The dramatic phenotype in the αERKO ovary appears to be secondary to the loss of ER\(\alpha\) function in the hypothalamus (Couse et al. 2003). Disruption of the ER\(\alpha\) gene results in very elevated LH serum levels due to a failure of the negative feedback action of estradiol on the hypothalamic-pituitary axis. The high levels of LH appear to be a major cause of the observed αERKO ovarian phenotype. Treatment with the gonadotropin releasing hormone antagonist antide suppresses the high serum LH levels and prevents the polycystic ovarian phenotype.

Ovaries from βERKO females show a different ovarian phenotype (Dupont et al. 2000; Krege et al. 1998). The βERKO ovary shows a normal gross morphology, containing all of the stages of folliculogenesis including corpora lutea. Upon superovulation with exogenous gonadotropins, however, βERKO ovaries have been observed to release a reduced number of oocytes compared with wild-type ovaries (Krege et al. 1998). Histological analysis of treated ovaries has revealed reduced numbers of corpora lutea and increased numbers of preovulatory follicles that had not released their oocyte. These data suggest that ER\(\beta\) may play a role within the ovary itself, possibly facilitating the ability of the ovary to respond to gonadotropins.

Adult αβERKO females exhibit an ovarian phenotype that is different from that observed in the individual knockout models (Couse et al. 1999; Dupont et al. 2000). Adult αβERKO female ovaries contain follicles, some of which have a large antrum but are devoid of corpora lutea. Surprisingly, these ovaries also contain tubule-like structures that resemble the seminiferous tubules of the testis. Additionally, the adult αβERKO ovary expresses mRNA of a specific Sertoli cell marker, Sox-9 (Couse et al. 1999). These tubules were not present in prepubertal mice even though they comprised a large portion of the adult ovary and lacked the granulosa cell layer typical of a maturing follicle. This observation leads to the hypothesis that these structures are redifferentiated follicles, rather than developmentally formed testicular cord-like structures (Couse et al. 1999). The observed loss of the oocyte in these follicles might trigger the cell reversal. Although other species have been described as having a morphological sex reversal of the ovary, the αβERKO is unique in that it is the first example of adult sex reversal (Couse et al. 1999). These results indicate that in mice, female somatic cells retain the capacity to redifferentiate to Sertoli-like cells throughout life. Thus, both ERs are required for ovarian function and oocyte survival in adults.

Mammary Gland

Mammary gland development is based on multihormonal control (Bocchinfuso et al. 2000), and development occurs in two major phases: ductal elongation during puberty, and lobuloalveolar development during pregnancy (Bocchinfuso et al. 2000; Korach et al. 2003). A rudimentary ductal system is present at birth near the nipple area. During puberty, the ductal system expands through proliferation at the terminal end buds of each branch. At pregnancy and with lactation, the entire system undergoes further branching in-
volving the formation of lobuloalveolar structures (Korach et al. 2003). Estradiol has been shown to stimulate the formation of terminal end buds directly and to stimulate cellular proliferation of the mammary ductal epithelium (Couse and Korach 1999a; Daniel et al. 1987).

ERα is highly expressed in the adult mouse mammary gland, whereas ERβ is only slightly detectable (Couse and Korach 1999b; Korach et al. 2003). The αERKO mammary gland exhibits normal prenatal and prepubertal development, but remains rudimentary after puberty, lacking the epithelial branching and lobuloalveolar development evident in wild-type glands (Bocchinfuso and Korach 1997; Korach et al. 2003) (Figure 1). Unlike the underdevelopment of the αERKO mammary gland, βERKO females possess normal ductal structure, and the entire fat pad is filled, as with wild-type females (Krege et al. 1998) (Figure 1). After pregnancy, βERKO mammary glands differentiate normally and reveal the lobuloalveolar structures necessary for lactation. The mammary gland of the adult αβERKO female resembles that of the αERKO female (Figure 1).

Both estrogen and progesterone are essential for ductal branching and lobuloalveolar development in the mammary gland. Because increased PR expression in the mammary glands depends on estrogen, the αERKO mammary gland phenotype might be at least partially due to the lack of PR induction. In αERKO mammary glands, basal PR mRNA expression is significantly reduced, and estrogen-stimulated increase in PR gene expression is lost (Bocchinfuso and Korach 1997). In addition to progesterone, prolactin (PRL1) is also necessary for full development of the mammary gland (Briskin et al. 1999). PRL mRNA has been observed to be significantly reduced in the αERKO pituitary, and PRL serum levels were lower in the αERKO (Scully et al. 1997). Therefore, the phenotype seen in the αERKO female mammary gland is likely to be due to a direct action of estrogens on the gland itself as well as indirect mechanisms involving the hypothalamic-pituitary axis. Data resulting from comparisons of the mammary glands of αERKO and βERKO female mice indicate that ERα is the main receptor necessary for mediating estrogen action in the mammary gland.

Phenotypes of the Male Reproductive Tract

Spermatogenesis

Male fertility and reproduction has been thought to be regulated by androgens; a possible role of estrogens in male reproduction remained controversial until the generation of the αERKO mouse model. There are no indications of defects in the development of the reproductive tract in αERKO, βERKO, or αβERKO male mice. Adult βERKO male mice are fertile and have shown no apparent or obvious morphological phenotypes (Krege et al. 1998). However, adult αERKO males are completely infertile when tested in timed-mating studies with wild-type females (Couse et al. 2001; Eddy et al. 1996). Further analysis of αERKO males has revealed abnormal spermatogenesis, reflected by lower sperm counts that continue to decline with age (Couse and Korach 1999a). Epididymal sperm collected from αERKO males have shown an increase in the incidence of sperm head separating from the flagellae and decreased sperm motility (Couse et al. 2001, Eddy et al. 1996). Testes of the αERKO male are somewhat smaller than those of wild-type males but contain the usual complement of seminiferous tubules. Histological analysis has indicated atrophy and dilation of the tubular lumen (Hewitt and Korach 2002) (Figure 2).

Impaired spermatogenesis is not caused by defects in the germ cells. Rather, it is caused indirectly through disruption in the somatic cells in the αERKO male reproductive tract. This characteristic has been shown by germ cell transplantation from donor αERKO male to the testes of wild-type recipients (Mahato et al. 2000). Thus, the disruption of ERα has resulted in serious impairments in both spermatogenesis and sperm function (Couse et al. 2001) (Figure 2). There were no abnormalities observed in the testes of βERKO male mice (Dupont et al. 2000; Krege et al. 1998) (Figure 2). αβERKO males show an overall testicular phenotype similar to the αERKO male (Couse and Korach 1999a,b; Dupont et al. 2000).

Accessory Sex Organs

Accessory sex organs such as the prostate, bulbourethral glands, coagulating gland, and seminal vesicle all are androgen dependent and serve to secrete components necessary for seminal plasma (Couse et al. 2001). In the rat, ER has been detected in various stages of development in each of the accessory organs mentioned (Cooke et al. 1991; Couse et al. 2001). Furthermore, ERα and ERβ are expressed in the prostate of various species (Couse et al. 1997, 2001; Hess et al. 1997; Lau et al. 1998). Although no apparent abnormality in the development of these accessory organ glands has been observed in the various ER knockout models, one striking observation in the αERKO male is a significant increase in the weight of the seminal vesicle/coagulating gland. This increase is more apparent with age and is most likely related to the slight increase in serum testosterone in the males (Couse et al. 2001).

Phenotypes of Nonreproductive Tissues

Estrogens and androgens are important for bone metabolism and homeostasis. Sex steroids are involved in bone modeling during adolescence and in the remodeling of bone in adulthood. Estradiol has been shown to be crucial for the maintenance of bone mass. Bone phenotypes observed in the ERKO mice show that femurs in αERKO and αβERKO mice are shorter than in wild-type mice, but not in βERKO
mice (Hewitt and Korach 2002; Vidal et al. 1999, 2000). It has been postulated that this characteristic is due to lower serum insulin-like growth factor-1 levels in these mice. Female αERKOs have smaller bone diameters and males have lower bone density (Couse and Korach 1999b). βERKO females have been reported to have an increase in bone mineral density content, and no changes have been observed in the βERKO males (Hewitt and Korach 2002; Windahl et al. 1999, 2001). These observations suggest that ERβ may have a negative effect on bone density in the female mouse (Hewitt and Korach 2002).

The protective factor of estrogen against cardiovascular disease in females may be due to their natural increased exposure to estrogens (Couse and Korach 1999a). An aortic injury model using the ERKO mouse models has analyzed the protective effects of estrogen on cardiovascular health. Those studies have indicated that ERα, but not ERβ, is needed to provide the protective estrogenic effects on vascular injury. Effects on reducing atherosclerosis and cholesterol levels were studied by crossing apolipoprotein E (ApoE<sup>−/−</sup>) mice with αERKO or βERKO. The crosses indicated that estrogen treatment reduces atherosclerosis in wild-type mice. These effects were absent in the αERKO/ApoE mice but still present in βERKO/ApoE mice, indicating a receptor-mediated role for ERα in this process (Hodgin et al. 2001).

**Summary**

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 and nonreproductive tissues. As described above, well-established phenotypes have been observed in various ERKO mouse lines. Future investigations using new mouse lines with modified estrogen receptors will allow investigators to continue evaluating the role of estrogen in normal physiology.

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**References**


Couse JF, Curtis SW, Washburn TF, Eddy EM, Schomberg DW, Korach...