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Receptor null mice reveal contrasting roles for estrogen receptor α and β in reproductive tissues

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1. Introduction

The biological effects of the steroid hormone, estradiol, are largely mediated by estrogen receptor (ER) proteins within the nuclei of target cells. The ER protein exists in two known forms, the ERα and ERβ. Both are thought to act as ligand inducible transcription factors and have therefore been categorized as Class I members of the superfamily of nuclear receptors [1]. Decades of study have revealed a plethora of roles fulfilled by the estrogen signaling system in the function of organ systems necessary for reproduction in the female. However, along with an increased understanding of the estrogen signaling system has come a greater appreciation for its role in additional organ systems, such as in the physiology of the skeletal and cardiovascular systems, as well as carcinogenesis and neurological illnesses.

Much of the current knowledge of the estrogen signaling system comes from in vivo laboratory animal studies involving ovariectomy or pharmacological treatments with antiestrogenic compounds and inhibitors of estradiol synthesis. These findings have been complemented by the vast knowledge gained from in vitro cell culture studies, employing chimeric and mutant versions of the ER, varied cell types, multiple combinations of promoter–reporter gene constructs, and the use of synthetic agonists and antagonists. However, there are distinct disadvantages to each of these experimental tools. Studies using aromatase inhibitors and/or estrogen antagonists are confounded by several factors, including variability of the compound to block the action of the natural enyzme or hormone. Furthermore, the effectiveness of various antagonists is highly dependent upon the animal model, the tissue or cell of study, the bio-availability of the compound at different target tissues, and the class of antiestrogen used [2]. This dilemma is further complicated by the discovery of the ERβ, since no known ER selective agonists or antagonists have been characterized at the in vivo level. The limitations of in vitro experimental approaches are obvious and based mostly on their finite application to the whole animal. As an alternative approach we have used gene targeting techniques to generate mice lacking either functional ERα or ERβ, and more recently, mice lacking both ERs, to provide a stable genetic model for evaluating estrogen receptor actions. The resulting estrogen receptor knockout (ERKO) models provide a unique tool to investigate the role of each ER in the context of the whole animal, and equally important, during the complete life-span of the animal. The loss of ERα leads to severe gonadal and behavioral phenotypes that result in infertility in both sexes of mice. In contrast, disruption of the ERβ gene results in subfertility in females whereas male fertility appears unaffected. This brief review will focus on a comparison of the reproductive phenotypes observed in these mutant animals and the contrasting roles for the two ERs that each model has revealed. A more comprehensive review of the varied phenotypes in the αERKO and βERKO mice has been recently published [3].

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2. Reproductive tract phenotypes of the ERKO female

In agreement with previous laboratory evidence indicating that estrogens are not required for differentiation and initial development of the female reproductive tract, the αERKO and βERKO female mice exhibit properly differentiated reproductive structures [4,5]. However, distinct phenotypes are exhibited in each model during adulthood, including a lack of sexual maturation of the gonadal ducts in the αERKO female and severe ovarian dysfunction in both the αERKO and βERKO females.

Sexual maturation of the female gonadal ducts requires more than the presence of ER but is marked rather by the acquired capacity to undergo the proper synchronized phases of proliferation and differentiation elicited by the ovarian-derived sex steroids. The uterine response to 17β-estradiol is a sequential process involving an array of metabolic and biochemical changes, including increases in water imbibition, vascular permeability and hyperemia, prostaglandin release, glucose metabolism, nucleic acid and protein synthesis, and ultimate cellular proliferation [6]. As shown in Fig. 1, the uteri of both adult αERKO and βERKO females develop normally and possess all three definitive uterine compartments, the myometrium, endometrial stroma, and epithelium (both luminal and glandular). However, estrogen resistance in the αERKO has led to severe uterine and vaginal hypoplasia (Fig. 1), resulting in uterine weights that are approximately half that recorded for wild-type littermates [4]. Estrogen insensitivity in the αERKO uterus is further illustrated by the complete lack of uterine growth and DNA synthesis when ovariectomized mice are treated with pharmacological doses of 17β-estradiol or the synthetic agonist, diethylstilbestrol (DES) [4,7,8].

Two genes known to be directly up-regulated in the uterus by the ER-estradiol complex via functional estrogen responsive elements are those encoding the progesterone receptor (PR) [9,10] and the secretory protein, lactoferrin [11]. We therefore utilized these estrogen markers to attest for estrogen insensitivity in the uteri of the αERKO mouse. A single dose of 17β-estradiol was highly effective in inducing the PR and lactoferrin genes within 24 h in uteri of wild-type mice but produced no such upregulation in the uteri of the αERKO mice, confirming the need for ERα in this response [8]. Interestingly, a recent report by Tibbetts et al. demonstrated that the estrogen-stimulated increases in PR are localized to the stromal and myometrial compartments of the uterus, whereas the increases in lactoferrin are isolated to the luminal and glandular epithelium [12]. Therefore, the loss of ERα function has resulted in estrogen insensitivity in all three anatomical compartments of the uterus.

The estrogen insensitivity in the uterus of the αERKO female is a contributing factor to the infertility of this mutant mouse, as the uterus is unable to prepare for pregnancy. In contrast, the uteri of adult βERKO females appear normal, undergo the cyclic changes associated with the ovarian steroid hormones [5] and exhibit a wild-type like uterotropic response when treated with exogenous estrogen agonists. Therefore, the estrogen responses necessary for sexual maturation and overall function of the uterus can occur in the absence of ERβ but are ablated by disruption of the ERα gene, congruent with ERα being the predominant ER expressed in the uterus [13,14].

A distinct advantage of null receptor models, whether naturally existing or experimentally generated via molecular methodologies, is their use as an *in vivo* tool for discerning alternate pathways of hormone action. Recent studies have indicated the preservation of a distinct estrogen signaling pathway in the αERKO uterus. Short-term treatments with the catecholestrogen, 4-hydroxyestradiol (4-OH-E_2), or the xenoestrogens, kepone and methoxychlor, result in similar increases in uterine weight and lactoferrin mRNA in both wild-type and αERKO mice [15,16]. Furthermore, the pure estrogen antagonist, ICI-182780, is unable to attenuate this uterine response in either wild-type or αERKO females, suggesting the presence of a non-ERα or –ERβ mediated signaling pathway for certain estrogenic compounds. This alternative pathway of lactoferrin gene regulation may involve activation of cAMP or growth factor response elements, both of which are present in the promoter region of this gene [17,18]. In light of the recent reports of local catacholestrogen synthesis in mammary tissue and the possible implications of these metabolites in breast cancer [19], further investigation into the alternate mechanisms by which these estrogens may activate nuclear processes is warranted.

As previously discussed, the PR gene is up-regulated in the uterus by the estradiol-ERα complex. Therefore, it was hypothesized that disruption of the ERα gene may subsequently result in abnormally low levels of PR in the αERKO uterus, and thereby render this tissue refractory to progesterone as well. However, basal levels of PR mRNA and protein in the αERKO uterus do not significantly differ from those in wild-type, although estrogen stimulated increases in PR levels are absent [8]. Furthermore, we have carried out a series of studies demonstrating the preservation of PR-mediated progesterone actions in the αERKO uteri, including (1) stimulation of the genes encoding amphiregulin and calcitonin, and (2) progesterone-induced decidualizaiton of the uterine stroma [20].

In contrast to the gonadal ducts, evidence of 17β-estradiol synthesis and estrogen receptors in the fetal ovary does suggest a role for estrogen in ovarian devel-

opment. A recent study has demonstrated immunohistochemical detection of both ERα and ERβ in the neonatal rat ovary [21]. Nonetheless, a lack of ERα or ERβ appears to have no gross effect on ovarian differentiation, since females of the individual ERKO lines possess normal ovaries at birth and during neonatal development [5,22]. However, at the commencement of sexual maturity, distinct ovarian phenotypes become apparent in the αERKO and βERKO females [5,22]. Continuous mating studies of sexually mature females indicate the βERKO females to be subfertile, defined as producing fewer litters of significantly lower numbers of pups, whereas αERKO females exhibit a complete inability to spontaneously ovulate and become pregnant. Therefore, the actions of both receptors are necessary for normal ovarian function, yet the pathways effected by the respective ER gene disruption most likely differ.

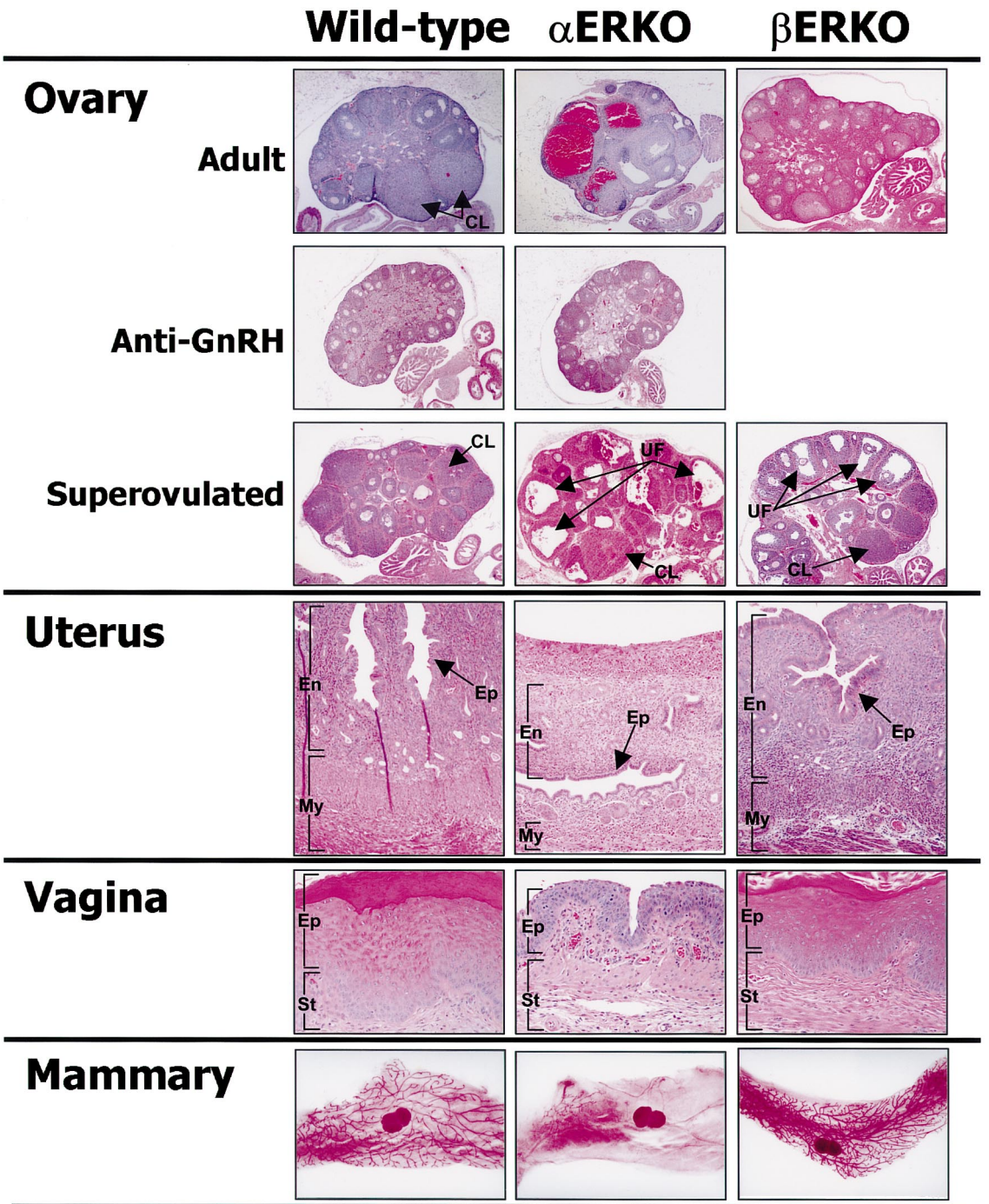

Fig. 1. *Caption overleaf.*

Concurrent with the endocrine functions of ovarian derived estradiol, such as the maintenance of the reproductive tract, mammary gland, and sexual behavior, are the reported intraovarian para/autocrine actions of estradiol. In 1940, Pencharz [23] and Williams [24] independently reported a direct and specific ability of estradiol or DES to induce significant increases in ovarian weight in the hypophysectomized rat. Since then, numerous intraovarian effects of locally synthesized estrogens have been described and postulated to be essential to ovarian function, including modifications in: (a) ER levels [25]; (b) DNA synthesis and cell proliferation [26–30]; (c) intercellular gap junctions [31]; and (d) follicular atresia [32]. Estradiol is also known to augment the actions of follicle-stimulating hormone (FSH) on granulosa cells, resulting in the maintenance of FSH-receptor levels [33,34] and the acquisition of LH-receptor [35,36], an event critical to successful ovulation. Ultimately, the intraovarian actions of estradiol act to enhance follicular responsiveness to gonadotropins, and thereby result in increased aromatase activity and further estrogen synthesis [36,37]. Studies of the localization of ERα and ERβ in the ovary indicate low levels of ERα in the thecal and interstitial cells, whereas ERβ is easily detectable and predominantly localized to the granulosa cells of growing follicles [14,21,22,38–40]. In addition, the dissimilar expression patterns for ERα and ERβ among the functional units of the ovary make unlikely any possible compensatory mechanisms fulfilled by the remaining functional ER in each respective ERKO ovary.

Therefore, given the many speculated intraovarian actions of 17β-estradiol, disruption of the respective ER genes may be expected to result in distinct ovarian phenotypes. The adult αERKO ovary is anovulatory and characterized by the accumulation of enlarged, hemorrhagic, and cystic follicles (Fig. 1). In contrast, the ovarian phenotypes of the βERKO adult female are more subtle, characterized by a relatively normal looking ovary but apparent infrequent and inefficient ovulation, as evident by the sparse appearance of corpora lutea and reduced fertility. In the ovaries of both mutants, growing follicles in the tertiary and pre- to small antral stage are present, indicating that neither ER is critical to the recruitment of primordial follicles and the initial stages of folliculogenesis. This is in contrast to the phenotypes of mice possessing a mutation of the steel (Sl/Sl^t) locus [41], or a targeted disruption of the genes encoding growth differentiation factor-9 [42], or the vitamin D receptor [43], all of which exhibit phenotypes of follicular arrest at very early stages.

Studies to characterize ovarian function in the ERKO females focused on immature animals, since the morphological ovarian phenotypes of ER disruption are not yet apparent. Superovulation with exogenous gonadotropins was successful in eliciting ovulation in immature females of both ERKO lines, however, the average number of oocytes collected from the αERKO females ($\sim$15 ooctyes/female) was significantly less than that yielded from age-matched wild-types ($\sim$40 oocytes/female) [5,44]. Interestingly, the superovulated βERKO females exhibited approximately 6 oocytes/female, a reduced number even when compared to the αERKO females. The ovaries of both ERKO mutant mice exhibited multiple ovulatory but unruptured follicles following superovulation treatment (Fig. 1). Therefore, the intraovarian actions of either ER are not obligatory to but appear to facilitate ovulation.

Follicular arrest, anovulation, or an attenuated response to superovulation, similar to that exhibited by

Fig. 1. Phenotypes in the reproductive tissues of the female αERKO and βERKO mice. [Ovary — Adult] Shown are ovarian cross-sections from representative adult wild-type, αERKO and βERKO females at low magnification. There is relatively little observable difference between the ovaries of the wild-type and βERKO mice, as both exhibit follicles at various stages of the follicular phase. In contrast, the αERKO ovary is characterized by the presence of large, hemorrhagic and cystic follicles, a sparse number of follicles at the early stages of proliferation, and a complete absence of corpora lutea. [Ovary — Anti-GnRH] Shown are ovaries from adult wild-type and αERKO females following prolonged treatment with a GnRH antagonist to reduce the elevated serum LH levels in the αERKO female to within the wild-type range (as described in Ref. [44]). This reduction in serum LH in the αERKO concurrently prevented the onset of the polycystic phenotype that occurs in the ovaries of all adult αERKO females, indicating this phenotype to be the result of chronic hyperstimulation of the ovary. Therefore, the polycystic phenotype of the αERKO is the result of the loss of ERα action within the anterior pituitary rather than the ovary. [Ovary — Superovulated] Shown are representative ovaries from immature wild-type, αERKO and βERKO females following a superovulation treatment with exogenous gonadotropins (as described in Refs. [5,44]). Ovaries from all three groups exhibit corpora lutea (CL), suggesting ovulation and terminal differentiation of the follicular cells. However, a multiple number of unruptured ovulatory follicles are present in the representative ovary of both ERKO mutants (indicated by arrows). [Uterus] Shown are cross-sections of uterine tissue from wild-type, αERKO and βERKO adult females illustrating the presence of the major anatomical tissue compartments, the myometrium (My) and endometrium (En), including the luminal epithelium (ep). Therefore, the loss of ERα or ERβ does not result in a disruption of proper uterine development. However, whereas the βERKO uterus is able to respond to ovarian steroid hormones and therefore appears much like the wild-type tissue, all functional compartments of the αERKO uterus are insensitive to estradiol and therefore severely hypoplastic. [Vagina] Shown are cross-sections of vaginal tissue from wild-type, αERKO and βERKO adult females illustrating the presence of the major anatomical tissue compartments the stroma (St) and epithelium (ep). As in the uterus, the loss of ERα or ERβ does not result in a disruption of proper vaginal development. However, once again, the βERKO vaginal tissue is able to respond to ovarian steroid hormones and therefore appears much like the wild-type tissue, whereas the αERKO vaginal tissue is insensitive to estradiol and therefore severely hypoplastic. [Mammary] Shown are whole mounts of mammary glands from wild-type, αERKO and βERKO adult females. There is relatively little observable difference between the glands of the wild-type and βERKO, as both exhibit the proper ductal network. In contrast, the αERKO gland is severely underdeveloped and appears similar to the gland of a neonatal female.

the ERKO mutants, have been reported in the ovaries of other murine models of gene disruption, such as mice lacking: (a) FSH [45]; (b) FSH-receptor [46]; (c) insulin-like growth factor-1 [47]; (d) cyclin-D2 [48]; (e) progesterone receptor [49]; (f) prostaglandin synthase-2 [50]; (g) connexin-37 [51]; (h) activin type II receptor [52]; (i) superoxide dismutase 1 [53]; or (j) Lats1 [54]. It is therefore possible that a loss of one of the ERs has also resulted in alterations in the expression and/or function of one or more of these gene products. However, recent studies in the αERKO ovary indicated that several of the above genes are properly regulated during superovulation [44]. Similar studies in the βERKO ovary are currently underway.

Some facets of ovarian physiology thought to be dependent on estrogen action are apparently preserved in the ovaries of the ERKO females. For example, although estrogen action is thought to attenuate granulosa cell apoptosis [32], abnormal levels of apoptosis are not evident in the ovaries of either αERKO [22] or βERKO females. Estradiol has also been shown to facilitate the FSH induction of LH receptors in the granulosa cells of the mature ovulatory follicle [35,36]. Nonetheless, the granulosa cells of the growing follicles as well as the enlarged cysts in the αERKO ovary possess significant levels of LH-receptor (LH-R) mRNA when assayed by in situ hybridization [22]. Similar localization assays for LH-R mRNA have not yet been carried out in the βERKO ovary, however, wild-type levels of LH-R mRNA have been detected in whole ovarian RNA preparations from βERKO females. Estradiol is also speculated to play a role in granulosa cell proliferation in the maturing follicle [26–30]. However, no marked differences in granulosa cell numbers are apparent in the follicles of the αERKO and βERKO ovaries.

A complete discussion of estrogen action and ovarian function must include the roles of estrogen in the hypothalamic–pituitary axis that are critical to folliculogenesis and ovulation. Gonadotropin synthesis and secretion from the anterior pituitary is at least partially regulated by gonadal steroids acting via classical feedback mechanisms in the hypothalamus and pituitary (reviewed in [55]). Indeed, disruption of the ERα gene has resulted in significant phenotypes in the hypothalamic–pituitary axis of the αERKO female, most notably in chronic hypersecretion of LH, resulting in serum levels that are four–seven times that found in wild-type females [44]. This dramatic phenotype is congruent with studies indicating that ERα is the predominant form of estrogen receptor in the mouse pituitary [13]. Biochemical evidence of hypergonadotropic-hyperstimulation of the αERKO ovary includes hypertrophied theca [22] and elevated levels of serum androgen and estrogen [3]. Furthermore, Risma et al. showed that increased serum LH levels attained via transgenic over-expression of the LHβ-subunit in the mouse leads to anovulation and multiple hemorraghic ovarian cysts, a phenotype almost indistinguishable from that of the adult αERKO female [56,57]. To determine the extent to which the αERKO ovarian phenotype was due to persistent stimulation by the heightened LH levels, we administered αERKO females a GnRH antagonist over the period during which the phenotype is known to develop and worsen. Prolonged treatment of αERKO females with a GnRH antagonist was successful in reducing serum LH levels to within the wild-type range and concurrently prevented the hemorrhagic and cystic ovarian phenotype, strongly indicating this ovarian phenotype to be the result of a lack of ERα within the hypothalmic pituitary axis rather than the ovary [44] (Fig. 1). This study illustrates the caution necessary when evaluating endocrine related phenotypes in the receptor knockout models since the inherent nature of endocrine hormone action requires the study of the whole animal rather than the immediate organ most obviously effected.

As in the αERKO, the ovarian phenotype of the βERKO female may also be due to altered gonadotropin synthesis and secretion from the hypothalamic–pituitary axis. The sex steroids also play an important role as a positive regulator of the preovulatory surge (reviewed in [58,59]). The ERα may be the predominant form of ER in the pituitary of the adult female mouse [13], however, both ERα and ERβ have been detected in various regions of the hypothalamus [60,61]. Preliminary data in the βERKO female indicate that tonic levels of serum LH are within the normal range. However, a lack of hypothalamic ERβ may have reduced the potential for positive regulation by estradiol in the hypothalamic–pituitary axis, and thereby may result in a reduction in the frequency and/or amplitude of the preovulatory gonadotropin surge necessary for ovulation. Nonetheless, the results of the superovulation studies described above, in which an artificial bolus of gonadotropin is administered to induce ovulation, indicate a severe intraovarian phenotype in the βERKO female.

3. Mammary gland

In mammals, the mammary gland is essentially undeveloped at birth and does not undergo full growth until the completion of puberty, and in fact, remains undifferentiated until pregnancy and lactation. Although estradiol is not essential to fetal mammary development, it is critical to maturation and differentiation of the gland during puberty and pregnancy, respectively [62]. Estradiol has been shown to directly stimulate the formation of terminal end buds and stimulate cellular proliferation of the mammary ductal epithelium [63]. Furthermore, this physiological effect can be inhibited

by antiestrogens [64], indicating a receptor-mediated pathway of estrogen action. The mammary glands of adult αERKO female mice possess the component structures necessary for mammary gland development but exhibit severly underdeveloped glands, appearing similar to those found in newborn females (Fig. 1). This phenotype strongly supports the need for ERα-mediated estradiol actions for mammary ductal growth [65]. Therefore, although embryonic and fetal development of the mammary gland in the mouse occurs in the absence of ERα, the pre- and postpubertal stages of growth in the mammary gland appear completely dependent on ERα action [65]. In contrast to the dramatic underdevelopment observed in the αERKO mammary gland, no such phenotype is observed in adult βERKO females. In our observations, virgin βERKO females of four–five months of age exhibit mammary glands possessing a normal ductal structure that fills the entire fat pad, and are indistinguishable from those of age-matched wild-type females (Fig. 1). This phenotype would appear to agree with our description of minor amounts of detectable ERβ mRNA in the adult mouse mammary gland, whereas ERα transcripts are easily detectable [13]. Furthermore, pregnant and nursing βERKO females possess mammary glands that have undergone normal differentiation to form the lobuloalveolar structures required for lactation. Therefore, the combined data from the αERKO and βERKO models indicate that ERα is likely to be the predominant receptor required to mediate the mitogenic actions of estradiol in the mammary gland of the mouse.

It is necessary to consider that the underdeveloped mammary gland of the αERKO female may be due to the direct as well as indirect loss of ERα actions. For example, progesterone action is critical to lobuloalveolar development, as confirmed by studies of the progesterone receptor knockout mouse, which exhibit normal pubertal glands but severe reductions in lobuloalveolar development [49]. Given the documented ability of the estradiol-ERα complex to increase PR expression in the mammary gland [66], a loss of ERα action may result in a loss of PR-mediated progesterone functions. Hence, we have reported reduced levels of PR mRNA in the mammary glands of adult virgin αERKO females, suggesting the possible attenuation of progesterone action in these tissues [67]. A likely indirect effect of the loss of ERα on mammary development involves secretion of the lactotropic hormone, prolactin. A similar phenotype of mammary gland underdevelopment is observed in female prolactin-receptor knockout mice, which possess a normal virgin mammary gland as adults, but severe deficits in lobuloalveolar development and lactation after pregnancy [68]. The synthesis and secretion of prolactin from the anterior pituitary is positively regulated by estradiol via ERα [69], as confirmed by a 20-fold decrease in prolactin mRNA in the anterior pituitary [70] and a five-fold decrease in serum prolactin levels in the αERKO female. Therefore, given the significant role of prolactin in the differentiation of the lactating mammary gland, it is likely that the phenotype described in the αERKO female is at least partially due to a lack of prolactin stimulation.

4. Reproductive tract phenotypes of the ERKO male

As expected in the presence of a functional androgen signaling system, the reproductive tracts in males of both lines of ERKO mice undergo prenatal development to produce normal internal and external structures. Furthermore, although Sertoli cells lining the seminiferous tubules of the testis produce 17β-estradiol [71] and express detectable levels of ER [72], estrogen action has been thought to play only a minor role, if any, in sperm production. Therefore, it was surprising to observe the severe impairments in spermatogenesis that contribute to complete infertility in the αERKO male. In contrast, studies in our laboratory have revealed no discernable deficits in spermatogenesis and fertility in young βERKO males; however, age-related effects are currently being evaluated.

Detailed investigations have demonstrated that infertility in the αERKO male is due to numerous effects resulting from disruption of the ERα gene, including significant reductions in sperm numbers, abnormal sperm function, and severe deficits in sexual behavior. As shown in Fig. 2, histological analysis of testes from sexually mature αERKO males indicate significant atrophy of the seminferous epithelium and severe dilation of the tubule lumen that worsens with age [73]. Further characterization of testes from mature αERKO males indicated a dilated rete testis that protrudes into the interior of the organ and severely dilated efferent ductules [73,74]. This phenotype suggests alterations in the critical fluid regulation function of the rete-testis and efferent ducts. Hess et al. have demonstrated that the reabsorption abilities of the efferent ductules in the αERKO male were compromised, while the secretory activity is actually reduced in the αERKO testis [74]. Further characterization indicated a reduction or often a complete lack of endocytotic vesicles and organelles common to fluid uptake in the epithelial cells lining the αERKO efferent ducts [74]. This study was the first report of a direct ERα mediated estrogen function in the male reproductive tract. Furthermore, preservation of ERβ expression in the αERKO strongly indicates that the reabsorption functions of the efferent ducts are indeed dependent on the presence of functional ERα. This view is strengthened by the lack of a similar testicular phenotype in βERKO male mice observed at ages as old as 14 months (Fig. 2) [5].

The accessory organs of the male reproductive tract include the prostate, bulbourethral glands, coagulating gland, and seminal vesicles. These glands have no known specific function other than to secrete components necessary to the volume of seminal plasma. All four tissues are dependent on androgen stimulation for growth and maintenance (reviewed in [75]). However, ER has been detected in each during various stages of development in the rat [76]. Furthermore, the prostate in various species appears to express significant mRNA levels for ERα as well as ERβ [13,77–79]. A series of studies by the Prins laboratory have described the toxic effects of neonatal DES exposure on the morphology and biochemistry of the rat prostate, including the regulation of AR, ERα and ERβ [80,81]. Nonetheless, we have observed no obvious abnormalities in the development of these glands in either the αERKO or βERKO mice studied to date (Fig. 2) [5,73]. However, more complete studies of morphological and biochemical markers of estrogen action in these tissues in both ERKO models are currently underway. In the αERKO, significant decreases in the weight of the epididymis/vas

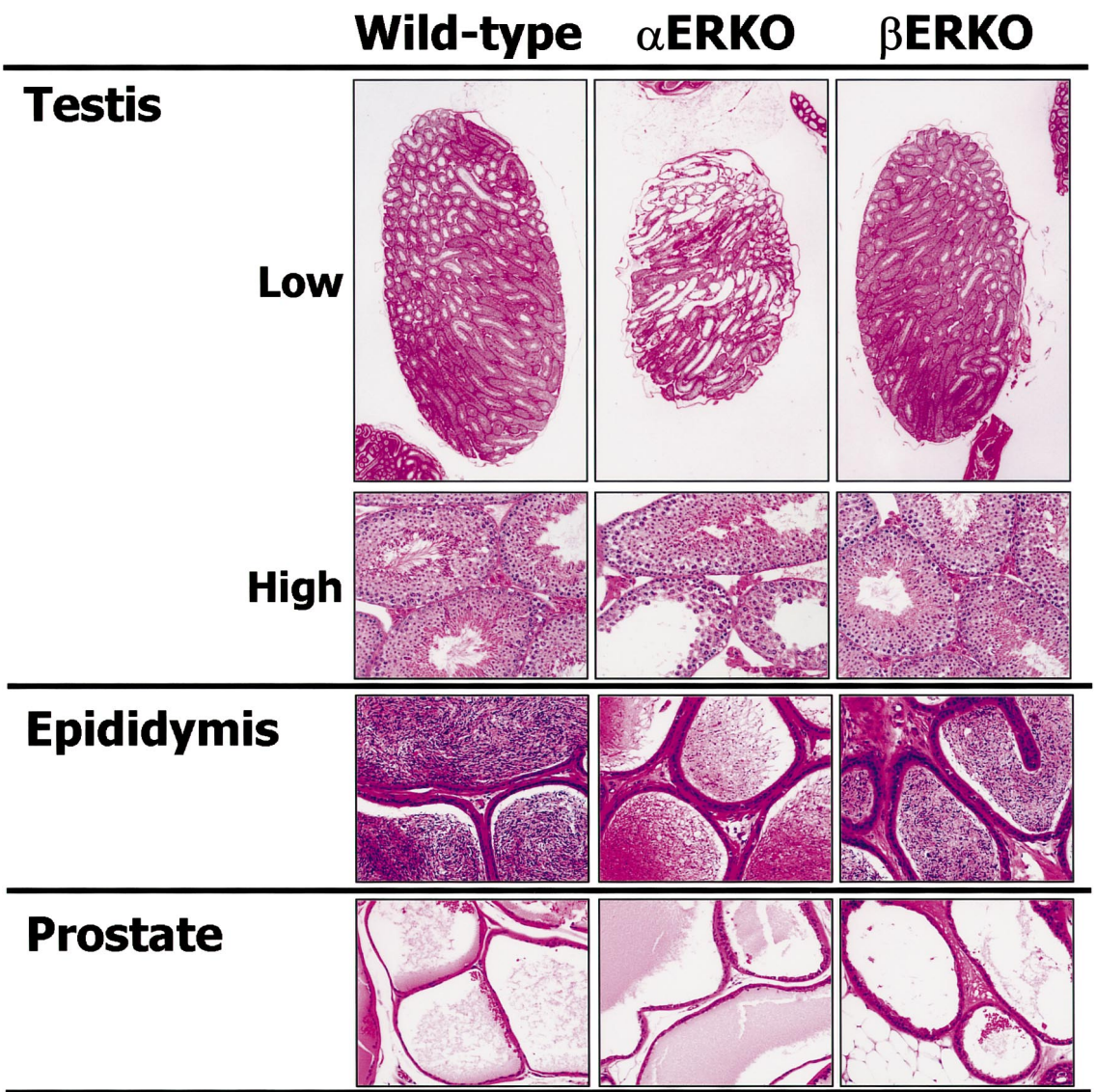

Fig. 2. Phenotypes in the reproductive tissues of the male αERKO and βERKO mice. [Testis] Shown are testis from representative adult wild-type, αERKO and βERKO males at Low and High magnification. There is relatively little observable difference between the testis of the wild-type and βERKO mice, as both exhibit normal morphology and ongoing spermatogenesis in the seminiferous tubules. In contrast, the αERKO testis exhibits severe dysmorphogenesis of the seminiferous tubules, characterized by a thinning seminiferous epithelium and a considerably dilated lumen. Spermatogenesis is disrupted, and excess fluid is present in the interstitium surrounding the tubules. [Epididymis] High-power magnification of the caudal epididymis from representative wild-type, αERKO and βERKO adult males, illustrating the reduced density in the population of epididymal sperm that occurs in the αERKO males only. [Prostate] High-power magnification of the prostatic tissue from representative wild-type, αERKO and βERKO adult males indicates no marked phenotypes in this tissue, despite the evidence of the expression of each ER in the mouse prostate.

deferens are observed, whereas the seminal vesicle/coagulating glands and prostate appear normal or often increased in size [73,82]. The decreased weights of the accessory organs are not due to the lack of necessary hormones, as serum levels of circulating gonadotropins and androgens are normal to slightly elevated in the αERKO.

5. Summary

The ERKO mice continue to illustrate the many ways in which the generation and study of knockout models can quickly contribute to the knowledge concerning the function of a particular gene product. However, it is worth noting that distinct differences in thought preceded the generation of each ERKO. At the time the work was initiated to generate the αERKO mice, there existed a substantial body of work concerning the many roles of estrogen-ER action, and hence, several educated predictions of the possible phenotypes were possible. Since the generation of the αERKO, a number of these predictions have been confirmed, rejected, or required re-evaluation. However, the generation of the βERKO mice occurred only two years after the discovery of the ERβ. Therefore, because so little was known about the function and role of the ERβ, it was difficult to make sound predictions. To date, the respective phenotypes in the αERKO and βERKO mice generally reflect the expression pattern of the two receptors throughout the body. In the αERKO, the most dramatic phenotypes occur in those tissues known to predominantly express ERα, such as in the uterus, mammary gland and pituitary. Likewise, the most dramatic phenotype in the βERKO occurs in the tissue that most heavily expresses the ERβ, i.e. the ovary. Certain other tissues that express both receptors do not exhibit a marked phenotype, such as the prostate, and will continue to require more study to determine the contributions of each ER form to the organ's function. In conclusion, the generation of the individual ERKO mice and our recently described 'double ERKO' or αβERKO [83], will prove invaluable in elucidating the precise roles fulfilled by each ER, as well as any possible cooperative roles the two receptors may play within the same tissue or even within the same cell.

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