Reproductive phenotypes in the estrogen receptor-α knockout mouse

Phénotypes de reproduction chez la souris knockout pour le récepteur-α de l’estrogène

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RÉSUMÉ - L’étude des souris privées de récepteurs alpha aux œstrogènes (ERαKO) est un modèle intéressant, bien que relativement nouveau dans le domaine de l’hormonologie. Ces souris seront d’une très grande utilité dans le futur pour mieux connaître le rôle des hormones stéroïdiennes dans le développement normal et la fonction endocrine. La souris ERαKO a été utilisée et continue de l’être pour déterminer le rôle des œstrogènes sur le système cardiovasculaire, le développement et la physiologie osseuse, le comportement, les réactions immunitaires, la neurophysiologie et la lipogénèse. On peut également espérer dans le futur des progrès considérables sur les effets de l’œstradiol et de la progestérone au niveau du cerveau, tout comme les effets impliquant des réactions croisées avec d’autres systèmes de signalisation. Enfin l’étude comparative du comportement des souris privées de récepteurs bêta aux œstrogènes, en combinaison avec l’analyse des souris ERαKO permettra de bien distinguer les différents rôles et les interactions pouvant exister entre les deux récepteurs dans le contrôle de l’effet des œstrogènes.

SUMMARY - The generation and initial characterizations of the ERαKO and has proved exciting, however, it is worth noting that this model is relatively new to the field. These mice will undoubtedly prove invaluable to future studies of steroid hormones in normal development and function. The ERαKO mouse has been and continues to be utilized to further study the role of estrogen action in the cardiovascular system [44, 25, 26, 22], bone physiology [30], behavior [40, 43], the immune system [49], neurophysiology [47, 48], and adipogenesis [51]. Furthermore, studies of the documented « non-genomic » effects of estradiol and progesterone in the brain [36] as well as ligand-independent actions involving cross-talk with other signaling systems will be advanced with further investigations using the ERαKO mouse. And finally, the ERαKO in combination with the recently developed ERβKO mouse [31] will prove invaluable in distinguishing the different roles that may exist between the two receptors in the estrogen signaling system.

INTRODUCTION

The biological effects of the steroid hormone, estradiol, are believed to be mediated in part by specific receptor proteins, now known to exist in two forms, estrogen receptor-α (ERα) and estrogen receptor-β (ERβ). Both are Class I members of the nuclear receptor superfamily, characterized as ligand inducable transcription factors composed of a highly conserved modular structure of functional domains [35]. As our understanding of the mechanisms of ER action grows, so does our appreciation for its role in several normal physiological processes. Estradiol is thought to play a role in the development and differentia-
Much of what is known about the role of estrogen and the ERα has stemmed from in vitro or in vivo studies utilizing ovariectomy or treatment with antiestrogenic compounds. Although these studies are of immense value, it is often difficult to infer from them the true events that occur within the complexity of the whole animal. Therefore, to further such investigations, we have employed the techniques of gene targeting by homologous recombination to disrupt both alleles of the endogenous ERα gene, generating a mouse completely devoid of functional ERα. This review will focus on the phenotypes in the reproductive tract that have been observed to date.

PHENOTYPES IN THE FEMALE

The uteri of adult ERαKO female mice are hypoplastic and weigh approximately half that of their wild-type littermates, but do possess all the characteristic tissue compartments of a normal uterus. The response of the rodent uterus to estradiol is occurs in distinct phases, with initial increases in water imbibition, vascular permeability, hyperemia, prostaglandin release, and protein synthesis [20], followed by dramatic increases in DNA synthesis, cellular proliferation, and hypertrophy within 24 hours of exposure [5]. When ovariectomized mice were treated with 17β-estradiol for three consecutive days, wild-type mice exhibited the expected 3-4 fold increase in uterine wet weight whereas no response was observed in the uteri of ERαKO mice [34]. These same results were obtained when mice were treated with the potent estrogen, diethylstilbestrol [28] or hydroxy-tamoxifen, an estrogen agonist in the mouse uterus [29]. The dependency on the presence of ERα for proper uterine cellular proliferation after estrogen exposure was confirmed by a [3H]-thymidine incorporation assay in which no significant increase in DNA synthesis over control animals was observed in the ERαKO compared to a 10-fold increase in the wild-type after a single estrogen treatment [6]. In addition to the lack of mitogenic actions of estradiol in the uteri of ERαKO mice, studies have also indicated the resistance in the regulation of known estrogen-induced genes. In the ERαKO mouse neither the genes for progesterone receptor or lactoferrin are up-regulated 24 hours after treatment with 17β-estradiol, confirming the need for functional ERα in this response [6].

A role for polypeptide growth factors, such as epidermal growth factor (EGF), as mediators of estrogen-induced mitogenesis in the rodent uterus have been indicated in several studies. This hypothesis is based on experiments demonstrating that in the uterus:
- estradiol up-regulates the uterine expression of both EGF and its receptor [10];
- treatment of mice with EGF mimics the effects of estradiol in terms of increased DNA synthesis, modified cell morphology, and lactoferrin expression [38];
- anti-EGF antibody was able to attenuate the response to estradiol [38];
- treatment with the estrogen antagonist ICI-164,384 was able to attenuate the response to EGF [23].

The conclusions drawn from these data has led to a model in which the mitogenic actions of estradiol in the rodent uterus appear to be at least partially mediated by EGF, however, in turn the mitogenic effects of EGF require the presence of ERα. We have recently shown that although the ERαKO uterus possesses normal levels of functional EGF and its receptor, it is unresponsive to the mitogenic actions of EGF, confirming the interaction between these two signaling systems [3]. However, not all EGF responses are negated in the uteri of ERKO females, as this same study demonstrated that the mechanisms for EGF mediated up-regulation of the c-fos gene remain intact in the ERKO [8].

The adult ERαKO ovary is characterized by the presence of primordial, primary, and secondary follicles but a consistent absence of ovulatory follicles and corpora lutea. Rather than proceeding through the stages of folliculogenesis to ovulation, the follicles in the ovaries of ERαKO mice appear to ultimately become arrested in a pre-antral stage and eventually become atretic, leading to the characteristic large, hemorrhagic, and cystic structures. This phenotype does not become apparent until after puberty and is therefore thought to be a secondary result of the hormonal imbalance that exists in the adult ERαKO female [45]. In normal physiology, subsequent increases in the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) into the circulating system lead to follicular maturation and ultimately to ovulation. We have demonstrated that removal of the ERα from the hypothalamic-pituitary axis has resulted in a disruption of the negative feedback actions of estradiol, resulting in increased secretion of LH [46]. Subsequently, the ovaries of ERαKO females undergo continuous stimulation, leading to an
anovulatory phenotype as well as a 10-fold increase in circulating levels of estradiol [6].

In support of this gonadotropin-based explanation of the ERαKO ovarian phenotype is a report in which Risma et al. [42] described a similarly hemorrhagic and cystic follicles in the ovaries of transgenic mice intended to overexpress LH. Furthermore, women demonstrating polycystic ovarian syndrome (PCOS) often present inappropriately high levels of circulating LH [16].

Other factors that may contribute to the ovarian phenotype of ERαKO females warrant consideration. Studies have demonstrated the presence of the ER in the granulosa cells of human [21] and rat ovaries [41], suggesting a more direct role of estrogen action in follicle development. Therefore disruption of the ERα gene may also negate the para/autocrine actions of estradiol in the ovary. In support of this hypothesis, an ovarian phenotype similar to that observed in ERαKO females was produced in rats after continuous treatment with the antiestrogens [12, 50]. Furthermore, studies of the rat ovary have shown that estrogen action is required to counteract the atretogenic effects of androgen [19]. This may be of significance since ERαKO females do not possess circulating levels of testosterone that are above the normal range. Interestingly, among several tissues analyzed in the rodent, the granulosa cells of the ovary possess the greatest concentration of ERβ mRNA [7, 32]. In fact, transcripts for ERβ are the predominant species of ER encoding mRNA in the adult wild-type mouse ovary [7]. These data would suggest that ERβ is the major receptor responsible for mediating the actions of estradiol in the ovary. However, despite the presence of relatively normal levels of ERβ mRNA in the ovaries of adult ERαKO mice, the loss of functional ERα results in the characteristic phenotype described above. Therefore, it is possible that the actions of ERβ are directly dependent on the presence of functional ERα or that a critical role for ERα precedes the functions fulfilled by ERβ during follicular maturation.

The mammary gland is essentially undeveloped at birth and does not undergo complete growth and differentiation until the onset of puberty, under the influence of both estrogen and progesterone, as well as growth hormone [24]. The mammary gland of an adult wild-type female mouse consists of a network of epithelial ducts originating from the nipple and forming a tree-like structure, each branch terminating in the form of an alveolar bud. At maturity, this ductal structure completely fills the fat pad that makes up the mammary gland. However, the mammary glands of mature ERαKO females demonstrate a simple rudimentary branch structure confined to the nipple region, resembling that of a normal female mouse prior to the onset of puberty. The lack of mammary gland development in the ERαKO is most likely the result of both the loss of direct as well as indirect actions of estrogen in this tissue. Although the sera of ERαKO females significantly elevated levels of estradiol as well as physiological levels of progesterone [6], no further development of the mammary gland appears with age, indicating resistance to both hormones. Furthermore, the local actions of peptide growth factors, such as EGF and transforming growth factor-α, are thought to be critical to proper mammary growth and development, but are also at least partially regulated by estrogen via the ER. And lastly, the anterior pituitary hormone, prolactin, is also thought to play a role in proper mammary gland development and function, but once again, estrogen is known to act as a positive regulator of prolactin production and/or secretion [1]. This may be relevant since pituitary secretions of adult ERαKO females have recently been shown to possess levels of prolactin mRNA that are nearly 20-fold lower than that of their wild-type counterparts [46]. Furthermore, we have carried out studies indicating that oncongene induced mammary tumors proliferate at a significantly decreased rate in the ERαKO female, indicating a role for Erα in the promotion of mammary neoplasias [4].

**PHENOTYPES IN MALE**

The phenotype of complete infertility in the ERαKO male as described by Eddy et al. was quite surprising [13]. As expected in the presence of a functional androgen signaling system, the reproductive tract of the ERαKO male grossly appears to have prenatally developed to produce the proper morphological structures. However, a report by Donaldson et al. [11] indicated a smaller yet more musculature in the cremaster sac and a greater incidence of the testes into the abdomen in ERαKO males compared to wild-type counterparts, suggesting a previously unrecognized role for the ERα in development of the male reproductive tract. Furthermore, sexually mature ERαKO males had a significant reduction in testicular weight compared to wild-type males, although changes in the epididymis, seminal vesicles, coagulating glands,
and prostate were not evident [11, 13]. Histological analysis of adult ER\textsubscript{α}KO testes revealed the presence of severely atrophied and degenerated seminiferous tubules, often possessing a dilated lumen and only a thin lining of Sertoli cells, as well as a disorganized seminiferous epithelium with few spermatogenic cells. This phenotype appeared to be age-related, commencing at approximately 10-12 weeks of age and progressing in a wave from the caudal pole toward the cranial pole of the testis. Suggested explanations for this phenotype have focused on the possibility of a disruption of the fluid transport mechanisms in the testis, resulting from a lack of normal cell-cell contacts, alterations in the cellular ion channels in the seminiferous epithelium, and an overall decreased rate of fluid reabsorption in the efferent ductules [13, 18].

Testicular steroidogenesis in the ER\textsubscript{α}KO does not appear to be greatly altered, nor do the circulating levels of LH and FSH [13]. These results provide strong evidence that steroidal feedback on the hypothalamic-pituitary axis in male mice is androgen/androgen receptor mediated and not dependent on aromatization of testosterone to estradiol and subsequent ER\textsubscript{α} actions [33]. Stermagenesis in the E.R\textsubscript{α}KO male appears to be present until approximately 10-12 weeks of age, when epididymal sperm concentrations decrease substantially, coinciding with the onset of the abnormal dilation of the seminiferous tubules described above [13]. However, the reproductive capacity of sperm from ER\textsubscript{α}KO males is significantly compromised even prior to the overall decrease in counts, exhibiting less vigorous motility and an overall decreased percentage of motile sperm compared to age-matched wild-types [13]. Furthermore, in vitro fertilization experiments using equal numbers of motile sperm from wild-type and ER\textsubscript{α}KO males demonstrated a complete failure of ER\textsubscript{α}KO sperm to fertilize [13]. Previous studies have suggested a role for the estrogen/ER pathway on Sertoli, spermatogenic, and epididymal epithelial cell functions [39], as well as in sperm transport and maturation within the epididymis [37]. These studies, combined with that of the E.R\textsubscript{α}KO, have provided evidence to support a requirement for ER\textsubscript{α} in spermatogenesis and fertility in the male mouse, however the exact mechanisms disrupted by the lack of ER\textsubscript{α} will warrant more detailed studies.

In behavioral studies, O'gawa et al. [40] have shown that a lack of hypothalamic ER\textsubscript{α} during development has little effect on mounting behavior and sexual motivation toward wild-type females in the mouse [40]. However, these same studies concluded that ER\textsubscript{α}KO males did exhibit an almost complete lack of intromission and ejaculation, even though the number and frequency of mounts were equivalent to those of wild-type males [40]. This behavioral phenotype is a contributing factor to the infertility of the E.R\textsubscript{α}KO male. Furthermore, ER\textsubscript{α}KO males were consistently less aggressive than their wild-type counterparts, exhibiting aggressive behaviors more typical of female mice [40].

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