Reproductive Functions of Progesterone Receptors

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ABSTRACT

The steroid hormone progesterone plays a central role in the reproductive events associated with pregnancy establishment and maintenance. Physiological effects of progesterone are mediated by interaction of the hormone with specific intracellular progesterone receptors (PRs) that are expressed as two protein isoforms, PR-A and PR-B. Both proteins arise from the same gene and are members of the nuclear receptor superfamily of transcription factors. Since these two isoforms were identified in the early 1970s, extensive controversy has existed regarding the selective contributions of the individual PR proteins to the physiological functions of progesterone. During the past decade, significant progress has been made in this regard using two complimentary approaches. First, analysis of the structural and functional relationships of each isoform using *in vitro* systems has generated compelling evidence to support the conclusion that PR-A and PR-B have different transcription activation properties when liganded to progesterone. Second, the advent of gene-targeting approaches to introduce subtle mutations into the mouse genome has facilitated the evaluation of the significance of observations made *in vitro* in a physiological context. Selective ablation of PR-A and PR-B proteins in mice using these technologies has allowed us to address the spatiotemporal expression and contribution of the individual PR isoforms to the pleiotropic reproductive activities of progesterone. Analysis of the phenotypic consequences of these mutations on female reproductive function has provided proof of concept that the distinct transcriptional responses to PR-A and PR-B observed in cell-based transactivation assays are, indeed, reflected in an ability of the individual isoforms to elicit distinct, physiological responses to progesterone. In PR-A knockout mice, in which the expression of the PR-A isoform is selectively ablated (PRAKO), the PR-B isoform functions in a tissue-specific manner to mediate a subset of the reproductive functions of PRs. Ablation of PR-A does not affect responses of the mammary gland or thymus to progesterone but instead results in severe abnormalities in ovarian and uterine function, leading to female infertility. These tissue-selective activities of PR-B are due to this isoform's ability to regulate a subset of progesterone-responsive target genes in reproductive tissues rather than to differences in its spatiotemporal expression relative to the PR-A isoform. More recent studies using PR-B knockout (PRBKO) mice have shown that ablation of PR-B does not affect ovarian, uterine, or thymic responses to progesterone but rather results in reduced mammary ductal morphogenesis. Thus, PR-A is both necessary and sufficient to elicit the progesterone-dependent reproductive responses necessary for female fertility, while PR-B is required to elicit normal proliferative responses of the mammary gland to progesterone. This chapter will summarize recent progress in our understanding of the selective contribution of the two PR isoforms to progesterone action.

I. Progesterone Receptor (PR) Isoforms

Receptors for progesterone are expressed as two distinct isoforms, PR-A and PR-B, that arise from a single gene (Conneely *et al.*, 1989; Kastner *et al.*, 1990). The expression of both isoforms is conserved in rodent and humans and overlaps spatiotemporally in female reproductive tissues. However, the ratios of the individual isoforms vary in reproductive tissues as a consequence of developmental (Shyamala *et al.*, 1990) and hormonal status (Duffy *et al.*, 1997) and during carcinogenesis (Brandon *et al.*, 1993; Graham *et al.*, 1996).

PRs have a modular protein structure consisting of distinct, functional domains capable of binding steroidal ligand, dimerizing liganded receptors, interacting with hormone-responsive DNA elements, and interacting with co-regulator proteins required for bridging receptors to the transcriptional apparatus (Guiochon-Mantel *et al.*, 1989; Tsai and O'Malley, 1994; Giangrande and McDonnell, 1999; McKenna *et al.*, 1999). Binding of progestin agonists induces conformational changes in receptor structure that promote interaction of coactivator proteins with distinct activation domains (AFs) located within both the amino- and carboxy-terminal regions of the receptor. Such coactivators promote chromatin remodeling and bridging with general transcription factors, resulting in the formation of productive transcription initiation complexes at the receptor-responsive promoter. In contrast, binding of receptor antagonist compounds induces receptor conformational changes that render AFs nonpermissive to coactivator binding and instead promote interaction with co-repressor proteins that inhibit the receptor's transcriptional activity. The ability of PRs to interact with a variety of coactivator and co-repressor proteins, together with the differing expression of co-regulators, illustrates a key role of these proteins in mediating different tissue-specific responses of progesterone receptors to steroidal ligand. Importantly, progesterone receptors also can be activated in the absence of steroidal ligand by phosphorylation pathways that modulate their interactions with co-regulator proteins (Rowan and O'Malley, 2000; Rowan *et al.*, 2000).

PR-A and PR-B isoforms differ in that the PR-B protein contains an additional sequence of amino acids at its amino terminus. This PR-B-specific domain encodes a third transactivation function (AF3) that is absent from PR-A (Sartorius *et al.*, 1994a; Wen *et al.*, 1994). Recent evidence has demonstrated that AF3 allows binding of a subset of coactivators to PR-B that is not efficiently recruited by progestin-bound PR-A (Giangrande *et al.*, 2000). Thus, when expressed individually in cultured cells, PR-A and PR-B display different transactivation properties that are specific to both cell type and target gene promoter context (Tora *et al.*, 1988; Meyer *et al.*, 1992; Vegeto *et al.*, 1993; Hovland *et al.*, 1998) and are associated with the differential ability of PR-A and PR-B to recruit specific co-regulator proteins (Giangrande *et al.*, 2000). Agonist-bound PR-B functions as a strong activator of transcription of several PR-

dependent promoters and in a variety of cell types in which PR-A is inactive. Furthermore, when both isoforms are coexpressed in cultured cells, in cell and promoter contexts in which agonist-bound PR-A is inactive, PR-A can repress the activity of PR-B. PR-A's repressor capability extends to other steroid receptors, including estrogen receptor alpha (ERα) (McDonnell *et al.*, 1994). Finally, PR-A and PR-B respond differently to progesterone antagonists (reviewed in Giangrande and McDonnell, 1999). While antagonist-bound PR-A is inactive, antagonist-bound PR-B can be converted to a strongly active transcription factor by modulating intracellular phosphorylation pathways (Beck *et al.*, 1993; Musgrove *et al.*, 1993; Sartorius *et al.*, 1994b). Although the ligand-binding domain sequences of PR-A and PR-B are identical, the ability of different ligands to induce different conformational changes in PR, together with the synergistic activity of the amino- and carboxy-terminal activation domains (Tetel *et al.*, 1999), predicts that PR-A- or PR-B-selective transcriptional regulation can be achieved by manipulating ligand interactions with the carboxy terminus.

II. Physiological Role of PRs

Null mutation of the PR gene encoding both isoforms has provided evidence of an essential role of PRs in a variety of female reproductive and nonreproductive activities. Female mice lacking both PRs exhibit impaired sexual behavior, neuroendocrine gonadotrophin regulation, anovulation, uterine dysfunction, and impaired ductal branching morphogenesis and lobuloalveolar differentiation of the mammary gland (Lydon *et al.*, 1995; Mani *et al.*, 1996; Chappell *et al.*, 1997,1999). PRs also play an essential role in the regulation of thymic involution during pregnancy and in the cardiovascular system through regulation of endothelial and vascular smooth muscle cell proliferation and response to vascular injury (Tibbetts *et al.*, 1999; Vazquez *et al.*, 1999). Progesterone receptors have been identified in the central nervous system and bone, where progesterone has been implicated in cognitive function and bone maintenance. However, the essential role of PRs in these regions has not been confirmed.

III. Generation of Mouse Models to Examine Selective Physiological Functions of PR-A and PR-B Proteins

Differences in transcriptional activities and co-regulator interactions between PR-A and PR-B observed *in vitro* predicted that these proteins may mediate different physiological responses to progesterone. In addition, the selective ability of PR-A to inhibit transcriptional responses induced by both PR-B and ERs suggested that PR-A has the capacity to diminish overall progesterone responsiveness in certain tissues as well as to contribute to anties-

trogenic activities of progesterone previously observed in the uterus. However, physiological validation of the functional differences between PR-A and PR-B isoforms has been hampered by a lack of information on the specific cell types that express each isoform *in vivo* and a dearth of appropriate animal models to determine their selective functions. The observation that PR-A and PR-B are produced by translation at two distinct AUG signals encoded by a single gene (Conneely *et al.*, 1987; Kastner *et al.*, 1990) predicted that mutation of either ATG codon in the PR gene would result in selective ablation of expression of a single isoform *in vivo*. Thus, we have used the CRE-loxP gene-targeting approach in embryonic stem cells to introduce a point mutation into the PR gene at the ATG codon encoding Met 1 (M1A) to specifically ablate expression of the PR-B protein and at the ATG encoding Met 166 (M166A) to ablate expression of PR-A. Consistent with previous findings *in vitro* (Conneely *et al.*, 1987; Giangrande *et al.*, 1997), we have demonstrated that these mutations are sufficient to ablate expression of the PR-B (PRBKO) or PR-A isoforms of PR (PRAKO) in mice (McDonnell, 1995) (Figure 1). The strategy provides a powerful model system to examine the selective expression of each isoform *in situ* as well as to assess the selective contributions of PR-A and PR-B in their normal cellular context to the physiological functions of progesterone.

IV. PRs and Ovarian Function

Evidence that ovary-derived progesterone may participate in autocrine regulation of ovarian function first emerged when it was demonstrated that

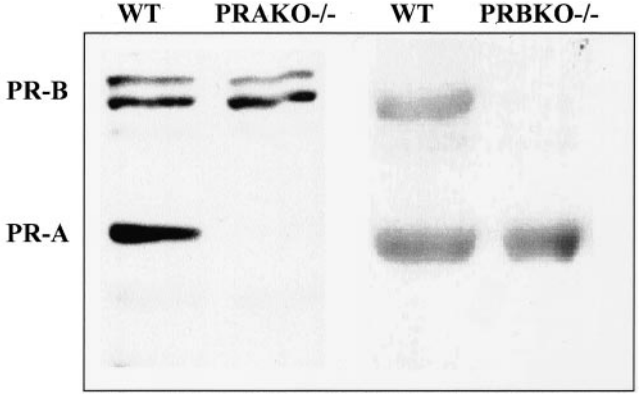

FIG. 1. Western immunoblot analysis to detect progesterone receptor (PR) isoform expression in uterine tissue extracts derived from wild-type (WT), PR-A knockout (PRAKO), and PR-B knockout (PRBKO) mice.

luteinizing hormone (LH), the primary signal for rupture of preovulatory ovarian follicles leading to ovulation, can stimulate transient expression of PR mRNA and protein in granulosa cells isolated from preovulatory follicles (Park and Mayo, 1991; Natraj and Richards, 1993; Park-Sarge and Mayo, 1994) and that the antiprogestin, RU486, can inhibit ovulation (Loutradis *et al.*, 1991). Definitive proof that PRs are essential mediators of ovulation has been provided through analysis of the ovarian phenotype of the PRKO mouse. Despite exposure to superovulatory levels of gonadotropins, PRKO mice fail to ovulate. Analysis of the histology of these mice has revealed normal development of intraovarian follicles to the tertiary follicular stage (Lydon *et al.*, 1995). The follicles contain a mature oocyte that is fully functional when isolated and fertilized *in vitro*. However, follicular rupture is effectively eliminated. Despite the ovulatory block, the preovulatory granulosa cells within these follicles can differentiate into a luteal phenotype and express the luteal marker, P450 side-chain cleavage enzyme (Robker *et al.*, 2000). Thus, PR is required specifically for LH-dependent follicular rupture leading to ovulation but not for differentiation of granulosa cells to form a corpus luteum (luteinization). Follicular rupture requires induction of a prostaglandin-mediated inflammatory response to LH as well as tissue degradation at the apex of the preovulatory follicle, an event that is mediated by matrix-digesting proteinases (Espey, 1994). Recent investigations to examine the molecular events associated with ovulation that are mediated by PRs have shown that PRs are induced specifically in the mural granulosa cells of the mature tertiary follicle and are absent from the cumulus granulosa cells that surround the oocyte (Robker *et al.*, 2000). Analysis of potential ovulation mediator expression in PRKO mice has demonstrated that LH-induced regulation of Cox-2, an enzyme that catalyzes prostaglandin production, is unaffected (Robker *et al.*, 2000). Cox-2 is required for ovulation and is expressed by cumulus granulosa cells (Dinchuk *et al.*, 1995; Morham *et al.*, 1995). In contrast, the expression of two metalloproteinases, ADAMTS-1 (a desintegrin and metalloproteinase with thrombospondin motifs) and cathepsin-L (a lysosomal cysteine protease) is inhibited in granulosa cells of mature follicles (Robker *et al.*, 2000) in PRKO mice. ADAMTS-1 recently was shown to play an essential role in ovulation (Shindo *et al.*, 2000), suggesting that this protein may be a critical mediator of the progesterone-induced ovulatory event.

Previous studies have indicated that both the PR-A and PR-B proteins are induced in preovulatory follicles in response to LH stimulation (Natraj and Richards, 1993). Therefore, to examine the selective roles of the individual PR isoforms in mediating the ovulatory function of progesterone, we have analyzed the ovulatory phenotype of mice in which expression of either the PRAKO or PRBKO isoform is selectively ablated. Stimulation of immature PRAKO mice with gonadotropins indicated that superovulation is severely impaired in these mice relative to their wild-type counterparts but, unlike PRKO mice, is not

completely absent (Figure 2). In contrast, superovulation was unaffected in PRBKO mice expressing only the PR-A protein. Thus, PR-A expression is both necessary and sufficient to mediate the ovulatory response to progesterone.

Histological analysis of the ovaries of PRAKO mice (Figure 2) showed numerous mature anovulatory follicles that contained an intact oocyte and were arrested at a similar stage to that previously observed in PRKO mice. Most surprisingly, however, in contrast to PRKO mice, progesterone's spatiotemporal regulation of ADAMTS-1 and cathepsin-L was unaffected. Thus, despite its inability to mediate follicular rupture, PR-B is functional in the ovary and capable of regulating a subset of progesterone-responsive target genes (B. Mulac-Jericevic and O.M. Conneely, unpublished data). The observation that the PR-A and PR-B proteins are not functionally redundant in the ovary provides physiological validation of previous studies in tissue culture demonstrating that these transcription factors have different functional activities. From a mechanistic standpoint, the observation that PR-A alone is sufficient to support normal ovulation indicates that heterodimeric interactions between PR-A and PR-B proteins are not required to regulate essential progestin-responsive target genes associated with ovulation. Using differential gene array approaches to identify differentially expressed genes in PRAKO and PRBKO mice should facilitate isolation of PR-A-selective target genes essential for ovulation as well as provide important new information on the molecular mechanisms of progesterone-induced follicular rupture.

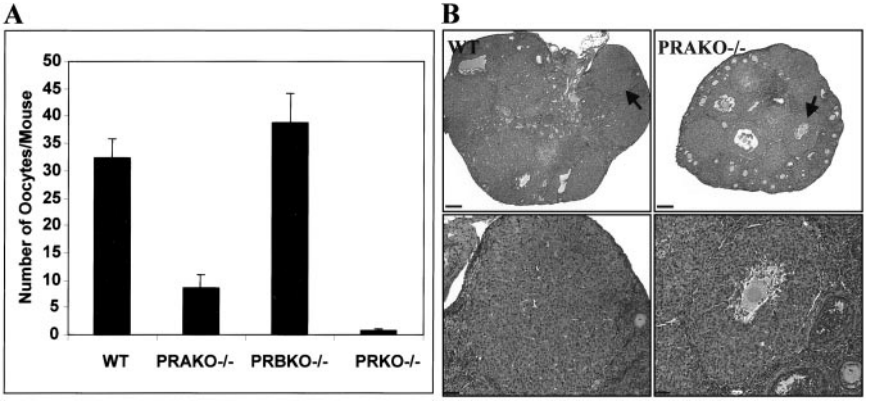

FIG. 2. Ovulation is critically dependent on PR-A function. (A) Average number of oocytes (± SEM) released per mouse after superovulation in WT, PRAKO-/-, PRBKO-/-, and PRKO-/- mice; n = 8 per test group. (B) Hematoxylin- and eosin-stained sections of ovaries from superovulated WT and PRAKO-/- mice. Scale bars = 200 μm. Higher magnifications of the ovarian structures indicated by the arrow are presented in the lower panels. Scale bars = 50 μm in the lower panels.

V. PR Isoforms and Uterine Implantation

Female infertility in PRKO mice also is associated with defective uterine implantation and a lack of decidualization of uterine stromal cells in response to progesterone (Lydon *et al.*, 1995). Consistent with these findings, wild-type embryos failed to implant when transferred into uteri of pseudopregnant PRKO females. Similarly, mating attempts between superovulated PRAKO females and wild-type males failed to result in successful pregnancies, despite the release of low numbers of oocytes from PRAKO females. To determine whether the PR-A protein is required for uterine decidualization, we treated ovariectomized mice with progesterone and estrogen, followed by mechanical stimulation of the left uterine horn of each animal (Ledford *et al.*, 1976), to induce decidualization of stromal cells. Decidualization is linked with a marked increase in uterine weight and a characteristic histological appearance associated with the differentiation of stromal cells into decidual cells. Both responses were inhibited in PRAKO mice, indicating that expression of PR-A in the uterus is required to mediate the decidualization response to progesterone (Figure 3).

To determine whether the decidualization defect in PRAKO mice was linked to aberrant regulation of progesterone-responsive target genes associated with implantation, we analyzed the expression of several implantation-specific uterine epithelial target genes. Specifically, we examined regulation of three genes: calcitonin (CT), histidine decarboxylase (HDC), and amphiregulin (AR) whose expression is increased in the uterine epithelium in response to progesterone in association with uterine receptivity (Das *et al.*, 1995; Paria *et al.*, 1998; Zhu *et al.*, 1998) and the epithelial secretory glycoprotein, lactoferrin (LF). LF expression is induced by estrogen and inhibited by progesterone (McMaster *et al.*, 1992). Progesterone-dependent regulation of each of these genes is abolished in PRKO mice. Ablation of PR-A, however, resulted in loss of expression of CT and AR, while the regulation of HDC and LF was fully retained (Mulac-Jericevic *et al.*, 2000). These findings indicated that defective implantation in PRAKO uteri is associated with loss of progesterone-regulated expression of a subset of genes involved with uterine epithelial receptivity. Importantly, this differential target gene regulation by PR-B was not due to differences in spatiotemporal expression of PR-B relative to PR-A. The expression of PR-B in PRAKO mice showed the same pattern of intrauterine expression and regulation by estrogen as that observed in wild-type mice (Mulac-Jericevic *et al.*, 2000). Thus, the uterine defects observed in these mice are due to differences in PR-B transcription factor activity rather than to differences in expression of the protein relative to PR-A.

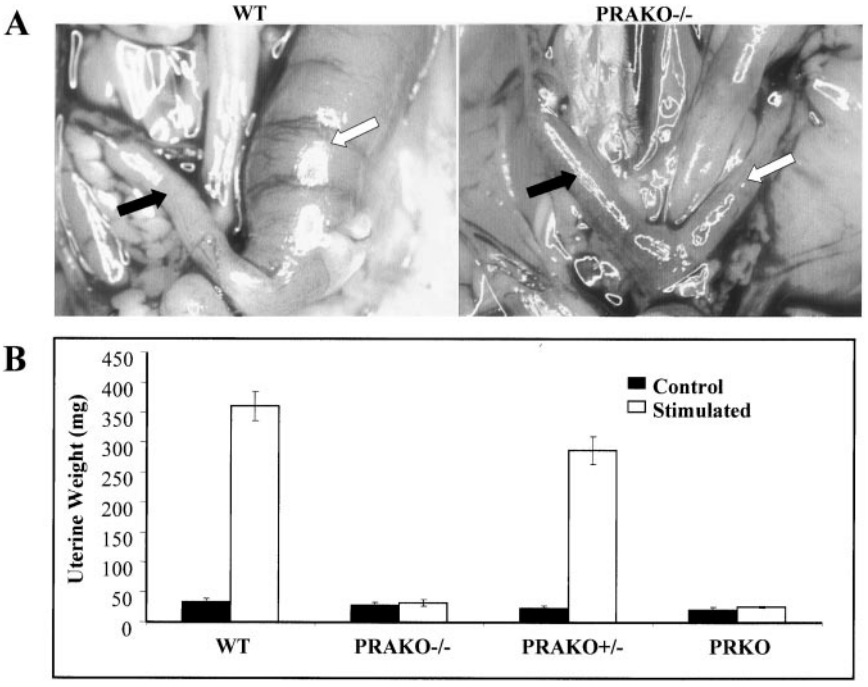

FIG. 3. The PRAKO uterus is unable to undergo decidualization. (A) Responses of the WT and PRAKO-/- uteri to mechanical decidualization. Black arrows indicate the unstimulated left uterine horn; white arrows denote the right uterine horn following stimulation. (B) Uterine weights following decidualization shown as means ± SEM; n = 6 per test group.

VI. Opposing Functions of PR-A and PR-B in the Regulation of Uterine Epithelial Proliferation

Estrogen is the primary proliferative stimulus for uterine epithelium. Its effects are inhibited by progesterone (Clarke and Sutherland, 1990; Lydon *et al.*, 1995). Ablation of both the PR-A and PR-B isoforms in PRKO mice results in marked hyperplasia of the lumenal and glandular epithelial tissue due to the unopposed action of estrogen (Lydon *et al.*, 1995). Selective ablation of PR-A, however, revealed an unexpected capacity of PR-B to contribute to rather than inhibit epithelial cell proliferation. Treatment of PRAKO mice with estrogen induced epithelial hyperplasia in a manner similar to that observed in PRKO and wild-type mice. However, progesterone plus estrogen resulted in a marked increase in proliferation over that observed with estrogen alone, a response that was not observed in PRKO mice (Figure 4). These results indicate that expression of the PR-B protein alone in the uterus results in a gain of proliferative

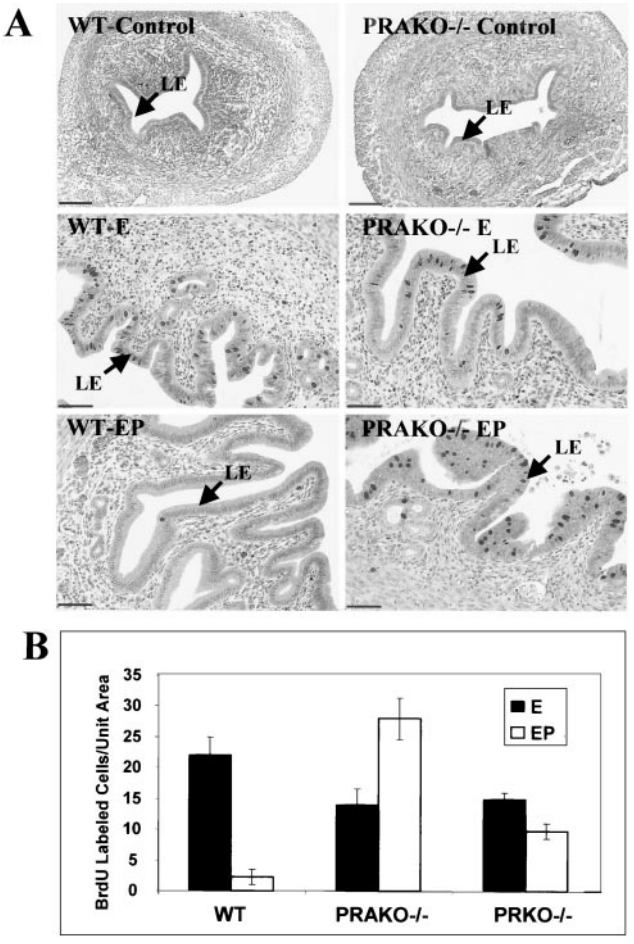

FIG. 4. PRAKO uterine epithelium exhibits abnormal proliferative responses to estrogen (E) and progesterone (P) treatment. (A) Bromodeoxyuridine immunolabeling of proliferating epithelial cells in uteri from ovariectomized WT and PRAKO-/- mice treated with sesame oil (control), E, or E + P (EP). Scale bars = 200 μm (controls) and 50 μm (hormone treated). LE, lumenal epithelium. (B) Quantitations of BrdU-positive uterine LE cells in WT, PRAKO-/-, and PRKO mice. The results presented are mean values ± SEM; n = 6 (WT and PRAKO-/-); n = 4 (PRKO).

activity (Mulac-Jericevic *et al.*, 2000). This acquisition of a proliferative activity of progesterone represents a PR-B-dependent gain of function not previously observed in the uterus and indicates that uterine expression of the PR-A isoform is required not only to oppose estrogen-induced proliferation but also that induced by progesterone acting through the PR-B protein. This dual role of PR-A

is of particular significance in light of previous studies carried out *in vitro*. Such studies have demonstrated that PR-A, when bound to progestin agonists, can inhibit target gene activation by both PR-B and ERα when tested in transfection assays using cultured cell lines in which agonist-bound PR-A alone is transcriptionally inactive (Vegeto *et al.*, 1993; Kraus *et al.*, 1995). These data predicted that PR-A has the capacity to diminish overall progesterone as well as estrogen responsiveness. Importantly, the inhibitory activity of PR-A observed in the uterus is tissue specific and is not observed in the mammary gland, where both estrogen and progesterone receptors contribute to ductal epithelial proliferation in the presence of PR-A.

The discovery that PR-B can contribute to rather than inhibit uterine epithelial cell proliferation is likely to have important clinical implications with regard to hormonal management of uterine endometrial dysplasias. Clearly, the relative expression of PR isoforms under these conditions will be an important determinant of the effectiveness of progestin therapy. Our results predict that progestin agonists selective for the PR-A protein should improve the effectiveness of progestin therapy in these conditions.

VII. Tissue-selective Regulation of Mammary Epithelial Branching Morphogenesis and Differentiation of Alveolar Lobules by the PR-B Isoform

Estrogen and progesterone are essential to maintain postnatal developmental plasticity of the mammary gland. Both hormones play a key role in mammary tumorigenesis. Null mutation of both PR isoforms in PRKO mice has demonstrated that PRs are specifically required for pregnancy-associated ductal proliferation and lobuloalveolar differentiation of the mammary epithelium. The mammary glands of PRKO mice failed to develop the pregnancy-associated side branching of the ductal epithelium with attendant lobular alveolar differentiation, despite normal postpubertal mammary gland morphogenesis of the virgin mice. Ablation of PR expression in these mice also resulted in a significantly reduced incidence of mammary tumor growth in response to carcinogen challenge (Lydon *et al.*, 1999). These observations underscore a specific role of PRs (as distinct from ERs) as obligate mediators of the intracellular signaling pathways essential for the initiation of murine mammary tumors induced by carcinogens.

Use of PRKO mice in combination with mammary gland transplantation techniques has provided important insights into the mechanisms underlying progesterone-dependent mammary gland morphogenesis. Throughout postpubertal mammary gland development, PRs are expressed exclusively in the epithelium (Seagroves *et al.*, 2000; Sivaraman *et al.*, 2001). Consistent with these observations, tissue transplantation approaches using wild-type and PRKO mouse tissue to produce mammary gland recombinants that were devoid of PR

in either the stromal or epithelial compartments have provided strong support for the functional involvement of epithelial rather than stromal PRs in mediating mammary gland morphogenic responses to progesterone (Brisken *et al.*, 1998). PR expression is localized to a scattered subset of epithelial cells throughout the ductal epithelium, most of which appear segregated from proliferating epithelial cells (Lydon *et al.*, 2000; Seagroves *et al.*, 2000). The hierarchical organization of these receptors and their segregation from proliferating cells are conserved features in rodent and human mammary tissue (Clarke *et al.*, 1997; Seagroves *et al.*, 2000; Sivaraman *et al.*, 2001). Such an expression pattern predicted that regulation of epithelial cell proliferation by progesterone may occur through a paracrine mechanism, whereby PRs residing in nonproliferating cells induce expression of a signal that promotes proliferation of neighboring receptor-negative cells. While PRKO mammary epithelium cannot undergo side branching, mixing experiments with PRKO and wild-type epithelial cells demonstrate that branching and differentiation defects can be overcome when PRKO cells are placed in close contact with PR-positive cells (Brisken *et al.*, 1998). Thus, although lacking PR-positive cells, the PRKO mammary epithelium retains those PR-negative cells that are responsive to PR-mediated paracrine signaling. Recent attempts to uncover downstream mediators of the progesterone response have identified the secreted glycoprotein, Wnt-4, as a potential PR target that is coexpressed in PR-positive cells, is regulated by progesterone, and is essential for regulating ductal branching via paracrine regulation of proliferation (Brisken *et al.*, 2000). Identification of additional paracrine mediators of the progesterone response using differential gene array analysis of PRKO and wild-type mammary glands is an imminently achievable goal.

In contrast to paracrine signaling pathways operative in the normal gland, in many breast tumors, the majority of ER- and PR-positive cells undergo proliferation (Clarke *et al.*, 1997). This observation would suggest that a switch in steroid-dependent regulation from a paracrine to autocrine mechanism may be an important part of the tumorigenic process. Consistent with this hypothesis, we recently observed that one of the earliest responses to carcinogen challenge in the mammary gland is the emergence of a population of proliferating cells that score positively for the expression of steroid receptors. This pattern is clearly at odds with the paracrine signaling pathways operative in the normal gland (Sivaraman *et al.*, 2001).

Both PR isoforms are expressed in the mammary gland of the virgin mouse (Shyamala *et al.*, 1990) and during pregnancy (Fantl *et al.*, 1999), although levels of PR-A exceed those of PR-B by at least a 2:1 ratio. To examine the selective contributions of each isoform to the morphogenic responses of the mammary epithelium to progesterone, we compared the morphology of mammary glands of ovariectomized, wild-type PRAKO and PRBKO mice after exposure to estrogen and progesterone. Ablation of PR-A in PRAKO mice did not affect the ability of

PR-B to elicit normal progesterone responsiveness in the mammary gland. The morphological changes in ductal side branching and lobular alveolar development in these glands were similar to those observed in wild-type mice (Figure 5) (Mulac-Jericevic *et al.*, 2000). Thus, the PR-B isoform is sufficient to elicit normal proliferation and differentiation of the mammary epithelium in response to progesterone. Neither process appears to require functional expression of PR-A. In contrast, more recent analysis of the mammary glands of PRBKO mice under similar conditions has shown markedly reduced ductal side branching and lobular alveolar differentiation (B. Mulac-Jericevic and O.M. Conneely, in preparation).

The PR isoform-selective morphogenic responses of the mammary gland observed in our studies do not phenocopy those previously observed when disruption of PR isoform ratios was achieved by overexpression of either PR-A or PR-B in the mammary glands of transgenic mice (Shyamala *et al.*, 1998,2000). PR-A overexpression caused mammary glands to exhibit increased ductal branching and hyperplasia and, most interestingly, abnormal disruption of basement membrane organization and decreased cell-cell adhesion (Shyamala *et al.*, 1998). In contrast, PR-B overexpression resulted in limited ductal elongation and branching, while alveolar growth was unaffected (Shyamala *et al.*, 2000). The striking differences in mammary defects observed in isoform-selective knockout mice relative to PR-A and PR-B transgenic mice could be explained by inappropriate targeting in transgenic mice of PR-A and PR-B expression to epithelial subtypes that normally would not express PR but may be competent to proliferate. Thus, indiscriminate targeting of these receptor isoforms to the mammary gland would breach the cellular segregation rules that apply to normal epithelial cell growth, resulting in a scenario reminiscent of the inappropriate colocalization of steroid receptor expression and proliferation observed in cells of breast tumors. Despite the phenotypic differences observed between the isoform-selective knockout and transgenic models, both approaches provide strong evidence that a regulated expression of both PR isoforms is critical for the mammary gland to respond appropriately to progesterone.

VIII. Summary

Over the past decade, significant progress has been made in understanding the collective and selective contributions of PR isoforms to the signaling pathways controlled by progesterone. Molecular dissection of progesterone signaling mechanisms using *in vitro* systems has demonstrated that the PR-A and PR-B proteins can respond to the same steroid ligand to induce both overlapping and distinct transcriptional responses that are promoter and cell context dependent. The use of genetically manipulated mouse models in which one or both of the PR isoforms is ablated has been pivotal in defining the physiological

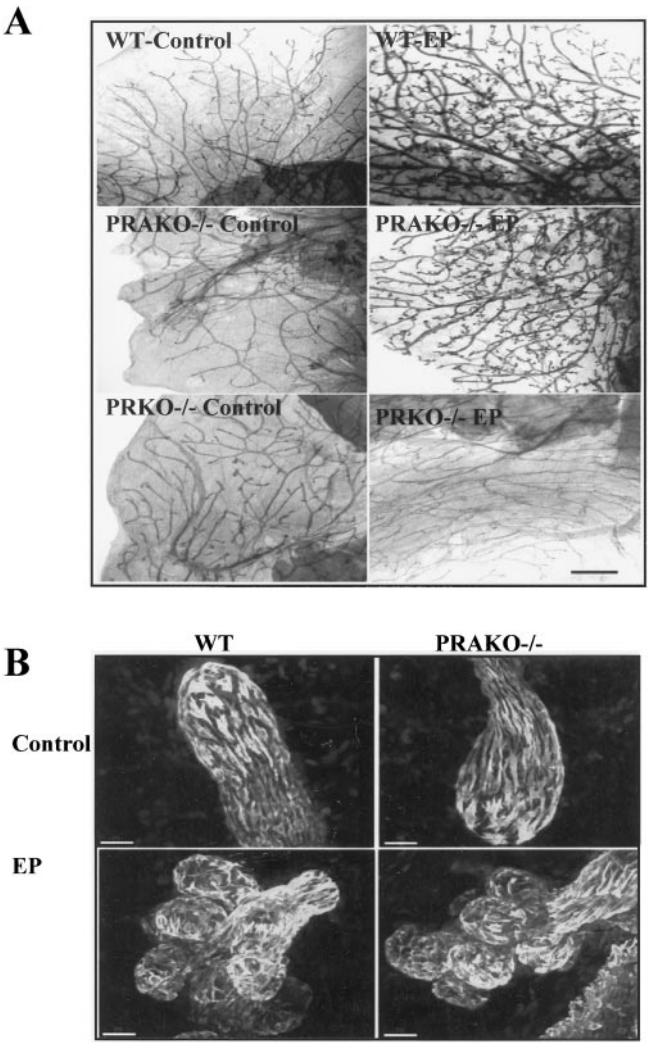

FIG. 5. The PR-B isoform mediates tissue-selective mammary gland tertiary ductal side branching and alveologenesis. (A) Thoracic mammary gland whole mounts of untreated (control) or E + P (EP) treated WT, PRAKO-/-, and PRKO mice. Scale bar = 500 μm. (B) Terminal ductal and alveolar structures visualized in the WT and PRAKO-/- mice using cytokeratin-14 immunofluorescence that specifically labels myepithelial cells. Scale bar = 20 μm.

spectrum of PR action as well as the contribution of individual protein isoforms to the pleiotropic activities of the hormone. These approaches have provided compelling evidence that the differences in transactivation properties of the PR

isoforms observed *in vitro* are reflected in a differential capacity to regulate the tissue-selective reproductive activities of progesterone. From a mechanistic standpoint, differences in physiological activities observed between PR-A and PR-B isoforms illustrate the key role played by amino-terminal AF domains in distinguishing tissue-specific responses to steroidal ligand. The results demonstrate that the inclusion or deletion of the N-terminal AF3 transactivation domain in PR is sufficient to alter tissue-specific responses to progesterone. Studies to date have selectively addressed the role of PR isoforms in mediating reproductive activities of progesterone. Genetic mouse models will provide valuable tools to address the physiological significance of ligand-independent pathways of receptor activation as well as the contribution of specific receptor subtypes to the activities of tissue-selective receptor modulators. In this regard, our observations predict that isoform-specific modulators of PR action should facilitate the identification of novel, tissue-selective modulators of PR-dependent reproductive functions.

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