Essential Role for Estrogen Receptor β in Stromal-Epithelial Regulation of Prostatic Hyperplasia


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Estrogens, acting via estrogen receptors (ER) α and β, exert direct and indirect actions on prostate growth and differentiation. Previous studies using animal models to determine the role of ERβ in the prostate have been problematic because the centrally mediated response to estrogen results in reduced androgen levels and prostatic epithelial regression, potentially masking any direct effects via ERβ. This study overcomes this problem by using the estrogen-deficient aromatase knockout mouse and tissue recombination to provide new insight into estrogen action on prostate growth and pathology. Homo- and heterotypic aromatase knockout tissue recombinants revealed stromal aromatase deficiency induced hyperplasia in normal prostatic epithelium due to disruption of paracrine interaction between stroma and epithelia. Treatment of tissue recombinants with an ERβ-specific agonist demonstrated that stimulation of ERβ elicits antiproliferative responses in epithelium that are not influenced by alterations to systemic androgen levels or the activation of ERα. Additionally, work performed with intact aromatase knockout mice demonstrated that the administration of an ERβ-specific agonist ablated preexisting prostatic epithelial hyperplasia, whereas an ERα-specific agonist did not. Therefore, failed activation of ERβ, resulting from local stromal aromatase deficiency, in conjunction with increased androgen levels, results in increased epithelial cell proliferation and prostatic hyperplasia. These data demonstrate essential and beneficial effects of estrogens that are necessary for normal growth of the prostate and distinguishes them from those that adversely alter prostate growth and differentiation. This highlights the potential of selective estrogen-receptor modulators, rather than aromatase inhibitors, for the management of dysregulated prostate growth. (Endocrinology 148: 566–574, 2007)

Numerous mammalian physiological systems in both males and females are regulated by androgens and estrogens, including reproductive tract function, development, and disease. In the male, androgens are generally considered to be proliferative, whereas estrogens have dual actions. In the prostate gland, estrogen has direct and indirect effects on epithelial cell differentiation and proliferation (1–3). Indirectly, estrogens suppress the release of pituitary LH, reducing synthesis of testicular androgens, which lowers systemic androgen levels and induces apoptosis and prostatic epithelial atrophy (4, 5). Concomitantly, acting locally via estrogen receptor (ER)α within the prostatic stroma, estrogens stimulate aberrant epithelial cell differentiation and proliferation, leading to squamous metaplasia (6, 7). More recently, an antiproliferative action of estrogen was suggested to occur via activation of the epithelial ERβ (8–10). However, those studies used intact wild-type (wt) or transgenic male mice, which remain sensitive to the inductive and instructive properties of the neonatal prostatic stroma, leading to perturbation of stromal-epithelial cell signaling and aberrant prostatic growth. In the absence of ERβ

Aromatase deficiency results in hypertrophy and hyperplasia of the prostate gland at maturity (11), providing a novel way of examining whether ERβ has antiproliferative activity within the prostate gland. Because aromatase is expressed in the stroma and ERβ receptors are located predominantly in the epithelia of the prostate (8, 12), we have proposed that the action of estrogen via ERβ involves stromal-epithelial cell signaling (13). Additionally, we believe that the action of estrogen via ERβ, like ERα, permanently and irreversibly alters prostate epithelial cell differentiation during development (6, 7).

Mesenchymal-epithelial cell interactions are vital for the correct development of the male reproductive tract, including the prostate gland (14). Tissue recombination techniques have successfully been used to define mechanisms of stromal signaling during development and differentiation of the epithelia, particularly the role of androgens acting via the stromal androgen receptor (AR) to induce epithelial differentiation and proliferation (15, 16). Taking a similar approach, we used prostate tissue from aromatase knockout (ArKO) mice to test the effect of a deficiency of prostatic stromal aromatase on prostate development and the subsequent activation of epithelial ERβ using a selective ERβ agonist.

This study shows that aromatase deficiency, and therefore the absence of local estrogen and failure to activate ERβ, has a significant impact on prostate development. It alters the inductive and instructive properties of the neonatal prostatic stroma, leading to perturbation of stromal-epithelial cell signaling and aberrant prostatic growth. In the absence of ERβ
signaling, increased cell proliferation results in non-neoplastic increases in epithelial tissue identified as epithelial hyperplasia (17).

This study demonstrates that although androgens are essential for the coordinated growth of the prostate, local estrogenic activity is equally essential for the modulation of normal prostate development. Estrogen, acting via ERβ, has a definite antiproliferative effect on prostatic epithelium that is able to prevent and ablate prostatic epithelial hyperplasia. Therefore, estrogens, acting in synergy with androgens and ERβ, are required to regulate the proliferative and antiproliferative changes that occur during normal prostate development and differentiation.

Materials and Methods

Animals

ArKO and severe combined immunodeficient (SCID) mice were housed at Monash Medical Centre under specific pathogen-free conditions with free access to soy-free chow and water. All experiments were approved by the Monash University Animal Ethics Committee and conducted in accordance with the National Health and Medical Research Council Guidelines for the Care and Use of Laboratory Animals. Day of birth was designated d 0.

Tissue recombination

Tissue recombinants were prepared as previously described (6, 14, 18) using newborn (d 0) wt or ArKO mouse stroma (S) and adult (16-wk-old) wt or ArKO epithelia (E). As indicated in Fig. 1, tissue recombinants composed of epithelial tissue from adult ArKO or wt prostates recombined with seminal vesicle mesenchyme (SVM) from newborn (d 0) ArKO or wt mice to produce the homotypic combinations wt-S/wt-E and ArKO-S/ArKO-E and heterotypic combinations ArKO-S/wt-E and wt-S/ArKO-E. Up to eight recombinants were grafted under the renal capsules of any one intact host SCID mouse. Because the genotype of neonatal mouse SVM donors was not available until after composition of the tissue recombinants and grafting into host mice recombinants was completed, the homo- and heterotypic grafts were randomly assigned to host mice, thus removing any bias in their positioning. After grafting under the renal capsule of intact male SCID mice, recombinants were harvested after 6 wk and fixed in Bouin’s fixative for analysis. A minimum of eight grafts were obtained for each tissue recombinant group.

Specific ER modulators

The ERβ-specific agonist (8β-ER2) and ERα-specific agonist (16α-Le2) were obtained from Dr. Fritzemeier (Schering AG, Berlin, Germany). The chemical characteristics and biologic effects of these compounds, demonstrating selective activation of ERs, have been previously described (19–21). Doses of ER-specific agonists used throughout this study were based on those previously used in male and female rats (19). ERβ agonist or placebo was administered to host SCID mice in the form of subcutaneous long-term release pellets (Innovative Research of America, Sarasota, FL) at the time of renal grafting, providing a dose comparable to 300 μg/kg d. In experiments using intact adult male ArKO mice, the ERβ agonist was administered by daily injection at doses of 100 and 30 μg/kg d. ERα agonist was administered to adult ArKO mice at doses of 3 and 0.3 μg/kg d by daily subcutaneous injection. In all cases, treatment with ER agonists was continued for 6 wk before tissue was collected. A minimum of four host SCID mice were used for treatment with either ERβ agonist or placebo to provide at least eight of each homo- or heterotypic tissue recombinant type. For treatment of intact animals, a minimum of six animals was used per treatment group.

Serum hormones

Measurement of serum testosterone levels was performed by ANZAC Research Institute (Sydney, Australia) using methods previ-
Immunohistochemistry

At 5 μm. Uniform systematic random sampling was used to select every 10th section for hematoxylin-eosin staining or immunohistochec- 

Quantitation of epithelial hyperplasia

Epithelial hyperplasia in tissue recombinants was identified accord-

Statistics

Data were analyzed to determine normality and significant differ-

Results

Estrogen deficiency alters the inductive and instructive properties of the neonatal stroma

Homotypic tissue recombinants. The regeneration of prostatic tissue using tissue recombination is based on the inductive 

To demonstrate the importance of locally synthesized estrogen on local stromal-epithelial cell interactions, we used tissue recombination to study the effects of estrogen defici-

Heterotypic tissue recombinants

The pivotal role of the stroma in the induction of epithelial hyperplasia was further demonstrated by comparing the heterotypic tissue recombinants (ArKO-S/wt-E, wt-S/ 

Overall, these data demonstrate that homotypic ArKO-S/ 

The data obtained from het-

Heterotypic wt-S/ArKO-E tissue recombinants show that the induc-

A further comparison was made of heterotypic wt-S/ 

The wt recombinants gave rise to normal prostate tissue with extensive epithelial infolding (Fig. 2B), characteristic of in vivo epithelial hyper-

ArKO-E recombinants (Fig. 2D) with homotypic recombi- 

Adult epithelial tissue (Fig. 1). The data obtained from het-

The data indicate that the absence of aromatase from the prostatic stroma, and thus estrogen synthesis, is necessary for the initiation of epithelial hyperplasia in mature (wt) 

A pivotal comparison between wt-S/wt-E and ArKO-S/ArKO-E (Fig. 1, C and D) with the homotypic recombinants. Epithelial hyperplasia was morphologically identifiable in ArKO-S/wt-E recombinants (Fig. 2C), being significantly greater than that seen in wt-S/wt-E recombinants and comparable to that seen in ArKO-S/ArKO-E grafts (Fig. 2E).

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perturbation in local stromal epithelial-cell signaling related to estrogen deficiency.

**Immunohistochemical localization of steroid hormone receptors and proliferation in tissue recombinants**

To further examine the local cell-cell signaling in these tissue recombinants, we investigated whether differences in hormone receptor expression could account for the different tissue responses to the same systemic hormone levels. The presence of AR is essential for normal prostatic differentiation (25) and initial examination identified AR immunoreactivity in all tissue recombinants; however, there were no obvious differences in the pattern of AR localization (Fig. 3, A–D). Although AR was localized to both stroma and epithelium, subsequent quantitation confirmed no significant differences in AR localization between tissue recombinants (Table 1). Localization of ERβ showed immunoreactivity was almost completely confined to the epithelial tissue of each graft (Fig. 3, E–H). Unlike ERβ, ERα could not be detected in any tissue recombinant nor was there any evidence of PR (data not shown).

Despite the absence of any obvious differences in the localization of steroid hormone receptors in the tissue recombinants, PCNA immunoreactivity was detectable in all tissue recombinants and was localized predominantly in the epithelium (Fig. 3, I–L). Quantitation of PCNA localization demonstrated significantly increased expression in all recombinants containing ArKO stroma and/or epithelium compared with the homotypic wt-S/wt-E controls (Table 2). Proliferation was significantly increased in wt epithelium after recombination with ArKO-S (ArKO-S/wt-E). In contrast, the proliferation in hyperplastic ArKO-E was unaffected by wt-S (wt-S/ArKO-E), exhibiting similar levels of proliferative activity to that seen in ArKO-S/wt-E and ArKO-S/ArKO-E recombinants (Table 2).

**Failure of stromal signals to stimulate ERβ activity results in induction of prostatic epithelial hyperplasia**

ERα and ERβ are believed to promote different proliferative responses in prostatic tissues. ERα is required for epithelial differentiation and proliferation (6), whereas ERβ is believed to be antiproliferative (8–10). However, the levels of androgens were not reported in these latter studies and if reduced would lower epithelial cell proliferation and induce apoptosis.

The results of the recombination experiments presented here did not show squamous metaplasia and implicate ERβ rather than ERα in mediating aberrant stromal-epithelial cell signaling leading to epithelial hyperplasia. In the absence of stromal aromatase activity and with reduced estrogen synthesis within the tissue, we postulated that a failure to locally activate ERβ caused epithelial hyperplasia in the tissue recombinants.

To test this postulate, we again used the aromatase-deficient prostatic tissue recombination model to reveal the cell-cell interactions, independent of systemic hormone levels. The tissue recombinants composed of neonatal stroma from ArKO mice (ArKO-S) with either wt or ArKO epithelium show epithelial hyperplasia so we administered an ERβ-
specific agonist to the host SCID mice bearing these homotypic tissue recombinants (ArKO-S/ArKO-E and wt-S/wt-E). Compared with placebo-treated controls (Fig. 4A), exposure to ERβ agonist had no obvious effect on the morphology of wt-S/wt-E tissues (Fig. 4B). However, compared with placebo treated ArKO-S/ArKO-E controls (Fig. 4C), treatment with an ERβ agonist abrogated epithelial hyperplasia in ArKO-S/ArKO-E recombinants (Fig. 4D) so that tissues were indistinguishable from wt-S/wt-E tissue recombinants. This inhibition of hyperplasia was also observed in heterotypic ArKO-S/wt-E and wt-S/ArKO-E tissues (data not shown). The total level of PCNA expression was significantly reduced in all ArKO-S/ArKO-E, ArKO-S/wt-E, and wt-S/ArKO-E tissue recombinants (Table 2). This significant response reflected changes observed in proliferation within the epithelial compartments of each of these grafts confirming the reduction in epithelial hyperplasia after exposure to the ERβ agonist was due to reduced proliferation of prostatic epithelial cells.

Proliferation was significantly higher in the stroma of wt-S/ArKO-E recombinants compared with that in wt-S/wt-E recombinants (Table 2) and is probably due to the reciprocal epithelial-stromal cell signaling from the hyperplastic epithelium. Treatment with ERβ agonist did not suppress proliferation in the stromal tissue of any recombinant consistent with ERβ being predominantly localized in the epithelium.

These data provide further evidence that the stimulation of epithelial ERβ is a major factor in regulating prostatic epithelial proliferation, but this occurs at a local tissue level and involves stromal-epithelial cell interactions within prostatic tissue. Although the systemic administration of ERβ agonist was able to ablate epithelial hyperplasia resulting from local deficiency in estrogen synthesis, it had no significant effect on homotypic wt/wt recombinants; neither did it alter the weight of SV, testis, or prostate lobes in the host mice (Table 3).

**TABLE 2. Quantitation of PCNA localization in tissue recombinants**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>% Tissue PCNA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epithelium</td>
</tr>
<tr>
<td>wt-S/wt-E</td>
<td>Placebo</td>
<td>16.2 ± 2.5a</td>
</tr>
<tr>
<td></td>
<td>+ERβ</td>
<td>17.7 ± 0.3a</td>
</tr>
<tr>
<td>ArKO-S/ArKO-E</td>
<td>Placebo</td>
<td>27.5 ± 2.5b</td>
</tr>
<tr>
<td></td>
<td>+ERβ</td>
<td>16.5 ± 0.8b</td>
</tr>
<tr>
<td>ArKO-S/wt-E</td>
<td>Placebo</td>
<td>28.9 ± 2.9a</td>
</tr>
<tr>
<td></td>
<td>+ERβ</td>
<td>18.9 ± 0.8a</td>
</tr>
<tr>
<td>wt-S/ArKO-E</td>
<td>Placebo</td>
<td>31.2 ± 3.8a</td>
</tr>
<tr>
<td></td>
<td>+ERβ</td>
<td>20.5 ± 0.4a</td>
</tr>
</tbody>
</table>

PCNA expression in placebo-treated ArKO-S/ArKO-E, ArKO-S/ wt-E, and wt-S/ArKO-E tissue recombinants was significantly elevated in epithelium and stroma compared with wt-S/wt-E recombinants. Treatment with ERβ agonist significantly reduced the percentage of cells proliferating in epithelium of ArKO-S/ArKO-E, ArKO-S/wt-E, and wt-S/ArKO-E tissue recombinants. Stromal proliferation was not significantly altered by ERβ agonist, but this lack of response did not significantly alter the total levels of proliferation apparent in each group of recombinants. Values indicate mean ± SEM, n = 8. Groups with the same superscript are not significantly different (P < 0.05).
matase deficiency. Thus, we tested the effects of administration of a specific ERβ agonist to intact ArKO male mice to determine if this selective ER modulator (SERM) could reverse existing prostate epithelial hyperplasia and/or hypertrophy. Over 6 wk of administration, the effects of the ERβ SERM were compared with those of an ERα-specific agonist.

Histologic comparison to wt tissue (Fig. 5D) showed that epithelial hyperplasia normally present in ArKO prostate (Fig. 5E) was attenuated and areas of ArKO tissue were morphologically indistinguishable from wt controls (Fig. 5F). In contrast, ArKO mice receiving an ERα agonist showed no attenuating influence on prostate hyperplasia (Fig. 5G) and exposure to this compound resulted in an inflammatory response evident from the infiltration of inflammatory cells into tissues, a response not seen in ArKO prostate tissue itself (data not shown). Neither ERα nor ERβ agonist showed significant effects on serum testosterone levels compared with control levels, although the responses were highly variable, as is commonly observed (Fig. 5A). Administration of both agonists reduced prostate weight (Fig. 5B), but only ERα agonist treatment reduced SV weight (Fig. 5C). The weight of the seminal vesicles is a good indicator of androgen levels and suggests the ERα agonist, rather than the ERβ agonist, lowered systemic androgen levels, although this was not detected in the serum assay.

![Fig. 4. ERβ agonist reduces epithelial hyperplasia in ArKO prostate tissue recombinants. A, wt-S/wt-E recombinant from vehicle-treated host showing normal epithelium. B, wt-S/wt-E recombinant from ERβ agonist-treated host showing normal epithelium. C, ArKO-S/ArKO-E recombinant from vehicle-treated host showing epithelial hyperplasia. D, ArKO-S/ArKO-E recombinant from an ERβ agonist-treated host shows loss of epithelial hyperplasia and is comparable to wt-S/wt-E control. Scale bars, 100 μm (A–D); 400 μm (insets).](https://academic.oup.com/endo/article-abstract/148/2/566/2501379)

**Table 3.** Weights of host mouse organs after ERβ agonist administration to tissue recombinants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body (g)</th>
<th>VP (mg)</th>
<th>AP (mg)</th>
<th>LP (mg)</th>
<th>DP (mg)</th>
<th>SV (mg)</th>
<th>Testis (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Placebo</td>
<td>53.2 ± 0.6</td>
<td>5.3 ± 0.4</td>
<td>27.8 ± 2.5</td>
<td>2.8 ± 2.3</td>
<td>8.4 ± 1.9</td>
<td>232.9 ± 81.8</td>
<td>149.1 ± 23.5</td>
</tr>
<tr>
<td>+ ERβ</td>
<td>53.5 ± 5.1</td>
<td>5.3 ± 1.5</td>
<td>25.6 ± 4.8</td>
<td>3.1 ± 0.1</td>
<td>7.1 ± 1.9</td>
<td>251.8 ± 63.8</td>
<td>152.3 ± 17.5</td>
</tr>
</tbody>
</table>

Administration of ERβ agonist (300 μg/kg per day) to intact male SCID mice bearing tissue recombinants did not significantly alter body weights or weights of SV, testis, ventral, anterior, lateral, or dorsal prostate lobes (VP, AP, LP, and DP, respectively) compared with placebo-treated SCID mice also bearing tissue recombinants. Values represent mean ± SEM, n ≥ 6 (P < 0.05).

**Discussion**

Estrogens exert both direct and indirect actions on prostate growth and differentiation. The indirect, centrally mediated response to estrogen administration (via ERα) suppresses androgen levels which in turn reduces epithelial cell proliferation. Consequently, this will mask any putative antiproliferative effects that may be mediated directly by ERβ. To overcome this, we have used the estrogen-deficient, ArKO mouse in addition to ERβ and ERα SERMs to unequivocally demonstrate that ERβ is a key factor in the regulation of prostatic epithelial proliferation and growth. This study reveals that a failure to activate prostatic ERβ leads to epithelial hyperplasia, is specifically the result of altered stromal epithelial cell signaling, is independent of systemic hormones, and is reversible after administration of an ERβ SERM.

Elevated levels of systemic estrogen in aging men are associated with the onset of benign and malignant prostate disease (27–29). Estrogen action, mediated via ERα, will cause aberrant cellular differentiation and proliferation with progression to prostatic hyperplasia, neoplasia, and dysplasia (1–3). Aromatase inhibitors have been tested as treatments for prostate disease, particularly cancer, yet have been surprisingly ineffective in reducing disease progression (30–32). Aromatase inhibition, however, will eliminate all estrogen action in the prostate, both direct and indirect, beneficial and adverse. Thus, any beneficial effects of estrogen acting via ERβ will be lost (8–10), which may account for the ineffectiveness of aromatase inhibitors in treating prostate disease. Therefore, suppression of estrogenic activity in the prostate may be detrimental, rather than beneficial, in combating prostate disease.

Unlike ERα, the stimulation of ERβ has been suggested to be antiproliferative. However, this effect has not been demonstrated independent of altered systemic androgen levels, which may also cause a reduction in proliferation. This study provides the first evidence that the ERβ-mediated effects on prostate epithelial cell proliferation are independent of systemic hormone levels. The absence of aromatase activity in both the stroma and epithelium of prostate tissue recombinants results in epithelial hyperplasia comparable to that seen in intact ArKO mice and in men with benign prostatic hyperplasia (17). These data demonstrate that the absence of local aromatase expression in the prostate is a key factor in determining epithelial cell hyperplasia. Based on our prior report demonstrating aromatase expression in the stroma, but not epithelia, of nonmalignant human prostate (13), it was assumed that the stroma was the major site of aromatase activity in the rodent prostate. Consistent with this assumption, tissue recombinants comprising wt epithelium and aromatase-deficient stroma (ArKO-S + wt-E) developed epi-
The ability of the stroma to direct epithelial development is not a new concept and has been studied and reported previously (34–37). Abnormal stromal characteristics lead to abnormalities in epithelial cell differentiation such as the lack of stromal AR expression contributing to aberrant epithelial cell differentiation during development or in cancer (34–37). The converse situation, in which normal stroma may reverse and/or abrogate an existing abnormal epithelial pathology, has not been reported nor was it observed in the current study. When normal stroma was recombined with hyperplastic ArKO epithelia (wt-S + ArKO-E), hyperplasia persisted. Although it is possible that a longer experimental time frame was required for the reversal of hyperplasia, this is unlikely. Additionally, Cunha and colleagues have demonstrated that the signaling between the stroma and the epithelium is reciprocal, and once deregulated, a vicious cycle of miscommunication ensues that serves to exacerbate the pathology (35, 36). This was readily apparent in the current study in which increased cell proliferation was observed in the stroma of tissue recombinants composed of wt stroma and ArKO epithelium (wt-S/ArKO-E).

The data presented provide new insight into the pivotal role of estrogen in stromal-epithelial cell signaling during prostate development. The disruption of stromal aromatase activity (and consequently estrogen synthesis) perturbs regulatory signals acting on the epithelium, resulting in epithelial hyperplasia that is independent of systemic hormone levels. However, the mechanism behind this effect is unclear. ERα induces atrophy via the suppression of androgen synthesis as well as promoting aberrant proliferation of the prostatic epithelium (squamous metaplasia). Loss or suppression of ERα activity, therefore, would be more likely to result in a normal epithelium than a hyperplastic one. Alternately, if ERβ is a negative regulator of prostate growth (8–10), then the loss of local estrogen metabolism would result in reduced ERβ activation and consequently increased cell proliferation. Consistent with this idea, the administration of an ERβ-specific agonist (ERβ SERM) inhibited the development of epithelial hyperplasia (ArKO-S/wt-E recombinants) and abrogated the existing hyperplasia in recombinants prepared with ArKO epithelia (ArKO-S/ArKO-E and wt-S/ArKO-E recombinants). Administration of the ERβ SERM also resulted in the ablation of existing epithelial hyperplasia in intact ArKO mice. Significantly, this occurred without any suppression of serum testosterone levels or other adverse affects on the hormone-sensitive tissues of the reproductive tract. This in vivo response is in direct contrast to the effects of an ERα agonist which, despite reducing the weight of the prostate and other reproductive organs, had no effect on the hyperplastic pathology of the epithelium.

These data strongly implicate ERβ stimulation as a major regulatory factor of prostatic epithelial cell proliferation. However, it is not yet clear exactly which endogenous ligands activate ERβ in vivo. It has been previously suggested that metabolites of reduced androgens such as 5α-androstan-3β,17β-diol (3βAdiol) may act as preferred ligands for ERβ, yet the results of the current study are inconsistent with this premise (9). The metabolism of testosterone results in both reduced androgens as well as estrogens via the reductase and aromatase enzymes respectively. As demonstrated, the failed ERβ activation in the ArKO mouse leads to the development of epithelial hyperplasia. Because reductase activity is not affected in this model (11), it would appear that locally synthesized estrogens are the preferred ligand for ERβ and are crucial for the prevention of epithelial hyperplasia. Although reduced androgens (like 3βAdiol) may bind to ERβ, they would appear to be much less effective in this role than estrogens.

Studies using exogenous estrogens have demonstrated both direct and indirect effects on prostatic differentiation and proliferation (Fig. 6A). Indirectly, estrogenic suppres-
information to the existing paradigm (Fig. 6B). First, it demonstrates an essential role for aromatase in stromal-epithelial cell signaling whereby locally synthesized estrogen activates ERβ to prevent epithelial hyperplasia. Second, it provides direct evidence that ERβ is antiproliferative without the complicating factor of altered systemic androgen levels.

This study clearly demonstrates that local estrogen synthesis is critical for normal growth of the prostate. Conversely, it is well known that too much estrogen has adverse effects on prostate pathology. More effective therapies will need to establish the right balance of estrogen, an outcome that cannot be achieved by aromatase inhibitors that block both the beneficial and adverse effects of estrogen via ERβ and ERα, respectively. This study suggests that ERβ-specific agonists may be of considerable benefit in the treatment of prostate disease together with antagonists to ERα.

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Disclosure Summary: V.P. and K.-H.F. are currently employed by Schering AG.

**References**

5. Thompson SA, Rowley DR, Heidger PJ 1979 Effects of estrogen upon the fine structure of epithelium and stroma in the rat ventral prostate gland. Invest Urol 17:85–89


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