REVIEW

Estrogen action on the prostate gland: a critical mix of endocrine and paracrine signaling

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Abstract

Although modern biotechnology has provided us with a greater understanding of the molecular events in endocrine-related diseases, such as benign prostatic hyperplasia and prostate cancer, these conditions continue to be a significant healthcare problem world-wide. As the number of men afflicted by these diseases will only continue to grow with the aging population, finding new strategies and new therapeutic options for the treatment of both of these diseases is crucial. A better knowledge of the mechanisms of hormone action is pivotal to making progress in the development of new hormone-based therapies. This is fundamental to increasing our understanding of the endocrine, paracrine, and autocrine signaling mechanisms in the prostate and in prostate disease, distinguishing the effects and role of each, and identifying where and how this communication goes wrong.

Journal of Molecular Endocrinology (2007) 39, 183–188

Endocrine signaling

As early as the 1940s, endocrinology has played a central part in the treatment of prostate cancer (PCa) when Charles Huggins and Clarence Hodges discovered a direct relationship between androgenic hormones and carcinoma of the prostate gland. Their seminal studies demonstrated that PCa was initiated by androgens while the growth of disseminated PCa could be inhibited by eliminating androgens (Huggins & Hodges 1941). This heralded the beginning of a new era for the treatment of PCa and this form of therapy, known as androgen ablation therapy (AAT), has been in use for decades and can involve castration or interference with the hypothalamic–pituitary–gonadal (HPG) axis.

AAT has undergone many permutations over the years; the overall objective of reducing androgen levels remains the same, but can be achieved in a variety of different means. Initially, AAT was achieved by surgical orchiectomy or by the use of oral estrogens in the form of diethylstilboestrol (DES). The use of oral estrogens for the treatment of hormone responsive PCa was based upon central feedback via the HPG axis with elevated estrogens leading to the suppression of pituitary gonadotropin secretion, resulting in the lowering of testicular androgen synthesis with subsequent prostatic involution and atrophy. However, deaths arising from estrogen-induced cardiovascular complications negated the survival benefit and estrogen-induced AAT was discontinued. More recently, this centrally mediated suppression of androgens has been facilitated by the use of luteinizing hormone–releasing hormone (LHRH) agonists (such as goserelin/Zoladex and leuprolide/Lupron), or LHRH receptor antagonists (such as abarelix/Plenaxis).

Despite the varied means in which it can be achieved, and while it remains the cornerstone of PCa treatment, AAT is not completely effective and essentially all ablated patients will eventually relapse. This relapse is also associated with progression of the disease to an androgen-independent state, for which there is no known treatment.

While the importance of androgens in the induction and progression of prostate malignancy is well accepted, the fact that estrogens are metabolites of androgens and can act locally is frequently unappreciated. Thus, estrogen action in the male must be viewed in at least two different ways: systemic endocrine effects acting via the pituitary to indirectly lower androgens and local paracrine effects that directly target prostate tissue itself. By developing an understanding of these mechanisms, we may be able to reduce our reliance on systemically mediated therapies for PCa and develop more effective and targeted treatment options.
Paracrine and autocrine signaling

Newer treatments for prostate disease have been based on regulating paracrine signaling within the tissue itself.

Paracrine signaling and mesenchymal–epithelial cell interactions are an essential component of androgenic control of the prostate gland (Cunha & Donjacour 1987) and AAT can also be effectively realized by targeting local androgen metabolism and signaling. Testicular testosterone is normally converted to the more potent androgen dihydrotestosterone (DHT) by the 5α-reductase type 2 enzyme (5αR) that is located in the prostatic stroma. DHT then acts in a paracrine manner to subsequently activate the epithelial androgen receptor (AR) to maintain secretory function. Treatment with 5αR inhibitors (such as finasteride) rapidly reduces DHT levels within the prostate, while AR antagonists (such as bicalutamide) effectively prevent any androgenic effects by blocking the androgen signaling pathway.

In a manner analogous, yet different, to androgens, estrogens also exert local effects in the prostate via paracrine mechanisms. An important difference is that estrogens are both adversely and beneficially implicated in the genesis and progression of PCa. This alters the application and complicates the use of estrogen-based therapies in the treatment of prostate disease.

Aromatase, the enzyme required for metabolism of androgens to estrogens, is expressed in the stroma of the normal prostate (Ellem et al. 2004). Thus, local estrogen signaling, which affects both epithelia and stroma, is paracrine in nature. Aromatase is aberrantly expressed in PCa, with induced expression and altered regulation in the epithelial tumor cells, resulting in a loss of paracrine (stromal–epithelial) regulation (Ellem et al. 2004) and the potential development of autocrine estrogen signaling within the epithelial layer.

Aberrant regulation and increased expression of aromatase plays an important role in the development and progression of malignancy in other hormone-dependent tissues, most notably the breast (Simpson et al. 1994). Given the putative adverse role of estrogen in the development of PCa, the aberrant regulation and increased expression of aromatase in PCa is significant and suggests estrogen blockade as a potential therapy.

Complete estrogen blockade (in a manner analogous to androgen deprivation via targeting 5αR activity) using aromatase inhibitors to treat PCa has been attempted in a number of prior studies, the results of which were surprisingly inconclusive. Initial trials reported that the administration of first and second generation aromatase inhibitors (e.g. aminogluthethimide) may be a valuable therapeutic option for patients with androgen-independent and/or advanced PCa (Rostom et al. 1982, Ponder et al. 1984, Kruit et al. 2004). However, additional trials using the third generation aromatase inhibitors (e.g. anastrozole, letrozole) proved to be unsuccessful, with few, if any, patients experiencing any objective response or disease stabilization (Santer et al. 2001, Smith et al. 2002).

The reason for the disparate results of these trials is uncertain, but it is clear that estrogen signaling in the prostate is very complex, owing principally to the presence of two receptor subtypes estrogen receptors α and β (ERα and ERβ). These receptors appear to play significantly different roles in the prostate, with ERα mediating the adverse and ERβ mediating the beneficial effects of estrogen.

Local actions of estrogen via prostatic ERα and ERβ

The role of estrogen within the prostate is a complex one that is particularly reliant on local signaling mechanisms in order to maintain a balance between the effects of ERα and ERβ. Estrogens are involved in local cell proliferation and prostate carcinogenesis in a manner analogous, yet different, to that of androgens. In addition, estrogens have also been implicated in the development of prostatic inflammation (Prins et al. 2001, Bianco et al. 2006).

Difficulty arises in distinguishing and delineating these separate roles, particularly as we have to consider signaling via endocrine, paracrine, and autocrine mechanisms. Tissue recombination is a powerful technique that allows us to distinguish between these mechanisms. The prostatic tissue recombinants are comprised stroma and epithelia from different ages or genetic strains of mice and are grown under the renal capsule of a host animal for several weeks to produce prostatic tissue (Cunha & Donjacour 1987, Cunha et al. 1991, Risbridger et al. 2001). This technique provides a means to assess the role and importance of cell–cell or stromal–epithelial cell signaling free from the confounding influence of altered systemic hormones, because different recombinants can be placed in the same host mice and exposed to the same endocrine hormones (Fig. 1).

Previously, the tissue recombination technique was used to successfully define the mechanisms of stromal signaling during development and differentiation of the epithelia, particularly in relation to the role of androgens acting via stromal AR and the induction of epithelial differentiation and proliferation (Cunha & Chung 1981, Thompson et al. 1986).
Proliferation

Estrogens play a role in proliferation in the prostate, but interestingly are capable of stimulating as well as inhibiting growth. This duality of action is specifically due to activation of each ER: ERα and ERβ.

Estrogens directly induce aberrant proliferation in the basal layer of the prostate epithelium. This causes a multi-layering of the basal cells and results in squamous metaplasia (SQM). Our previous studies using tissue recombination have demonstrated that this response is dependent on local paracrine signaling mechanisms, with ERα expression in both the stroma and epithelium being required to produce this proliferative response (Risbridger et al. 2001). The induction of SQM by estrogens is significant for two reasons. First, the proliferation is aberrant and contrasts to the ordered and co-ordinated proliferation that is induced by androgens. Secondly, as this proliferation is aberrant, it may eventually progress and lead to further prostate pathologies such as inflammation and/or cancer.

In addition to promoting aberrant proliferation and SQM, estrogens are also anti-proliferative. The aromatase knock-out (ArKO) mouse is estrogen deficient and develops prostatic hyperplasia and hypertrophy (McPherson et al. 2001), both of which are suppressed and/or ablated in intact animals following the administration of an ERα-specific (but not ERβ) agonist (McPherson et al. 2006).

In order to confirm this role of ERβ and remove and eliminate any possible systemic influence, demonstrating the importance of local ERβ activation, we utilized tissue recombination again. When tissue recombinants composed of stroma and epithelia from estrogen-deficient ArKO mice were grown in host male mice with normal endocrine hormone profiles, the prostatic tissue still developed hyperplasia. Furthermore, this pathology was abrogated following ERβ agonist administration (McPherson et al. 2006). These data conclusively demonstrate that a failure to activate ERβ, locally and within the tissue itself, will result in the development of prostatic hyperplasia and is independent of the systemic hormone status.

Inflammation

A pivotal role for estrogen in prostate inflammation is evident from our previous studies on estrogen action using the hypogonadal (hpg) and ArKO mouse models. When exposed to estradiol for 6 weeks, we have shown that mature hpg mice demonstrate a proliferative response within the prostatic stroma and epithelium (Bianco et al. 2002). In addition to this response, neutrophils identified in the stroma were shown to migrate through the epithelium to the lumen which is distended as a result of accumulated cellular debris comprising epithelial cells, inflammatory cells, and anuclear keratinized deposits (Bianco et al. 2002). The hpg mice are completely deficient in pituitary gonadotropins and, subsequently of sex steroids, so there are no confounding effects of the androgen withdrawal due to ‘chemical castration’, and thus the inflammatory pathology must be a direct response of the tissue to estrogen. The estrogen-deficient ArKO mouse model is unhindered by complications arising from normal endogenous estrogen synthesis and clearly show the effects of transient elevation of estrogen. When we transiently treated ArKO mice with DES in neonatal life, prostatic epithelial dysplasia and inflammatory cell infiltrates (comprised neutrophils and lymphocytes) are observed in the ventral and dorsolateral prostate lobes upon aging (Bianco et al. 2006).

Further support for a role of estrogen in the induction comes from the aromatase over-expressing (AROM+) mouse. Preliminary data from our laboratory have revealed that these mice develop extensive...
inflammation of the prostate during late life (Ellem et al. unpublished observations).

The inducement of inflammation by estrogens is specifically mediated by ERα (Prins et al. 2001). ERα knock-out (zERKO) mice exhibit no such prostatic response to neonatal DES but βERKO mice show a response similar to that observed in wt animals (Prins et al. 2001). Thus, ERα is the dominant ER subtype mediating the inflammatory response to estrogenization of the prostate gland. Although it has been suggested that elevated prolactin may be involved in the inflammatory response in rats (Tangbanluekal & Robinette 1993), this is unlikely to be the case in mice as ArKO mice do not develop inflammation in the absence of estrogen, despite life-long elevated levels of prolactin.

Although estrogens, acting via ERα, have been shown to induce inflammation, ERβ has also been suggested to have anti-inflammatory effects. A number of animal models of diseases like bladder cystitis, inflammatory bowel disease, and microglia have reported possible beneficial effects of ERβ-specific agonists on inflammation. Additionally, genistein (a naturally occurring selective estrogen receptor modulator with a high affinity to bind ERβ in vitro) was recently shown to reduce the production of pro-inflammatory molecules in human chondrocytes (Hooshmand et al. 2007).

Despite these data, very little is known about the role of ERβ in prostatic inflammation. However, there is a significant volume of compelling evidence supporting a role for inflammation in the pathogenesis of PCa (De Marzo et al. 1999, 2007, Nelson et al. 2004, Palapattu et al. 2005), and therefore the link between estrogens, inflammation and PCa warrants investigation.

**Carcinogenesis**

Induction of PCa requires the combined actions of both T+E, with work in our laboratory and elsewhere indicating that although both androgens and estrogens have the potential to initiate changes in the prostate independently, individually they cannot produce malignancy (Risbridger et al. 2003).

The Noble rat model is frequently used to study the effects of combined hormone treatment on PCa. When administered to these animals, T+E induce premalignant prostatic intraepithelial neoplasia (PIN) lesions that are similar to those described in men, as well as stimulating prostatic adenocarcinoma (Leav et al. 1989). More recently, this effect was also demonstrated in mice (Ricke et al. 2006). Rodents do not spontaneously develop PCa, and this is one of the few means capable of inducing PCa in these animals that does not rely on some form of genetic modification or SV40T antigens.

Combined hormone treatment to mature mice resulting in carcinoma has yielded interesting new insights to the hormonal induction of carcinogenesis and definitively shown that ERα mediates this response. The PIN lesions observed in T+E-treated animals are characterized by significantly elevated ERα expression within the lesion itself. Significantly, PIN lesions can be induced in βERKO, but not in αERKO, mice following T+E treatment (Ricke et al., unpublished data).

Also of note is that when the estrogen-deficient ArKO mice are treated with the same regime, the incidence of PIN lesions is lower than seen in wild-type mice. This latter observation is further evidence that the absence of estrogens, and thus signaling via ERα, is beneficial, at least in this context.

**Figure 2** Summary of local estrogen signaling mechanisms. Schematic of the local estrogen signaling mechanisms in the prostate gland. Testosterone is locally metabolized to estrogen, by the aromatase enzyme and acts via ERα or ERβ. Adverse effects via ERα in the stroma and epithelia include aberrant proliferation, inflammation, and eventually, cancer. In contrast, estrogen is also able to exert beneficial effects via ERβ in the epithelia, which has been implicated in preventing hyperplasia and hypertrophy, being anti-proliferative and, potentially, anti-carcinogenic.
There are also clinical and biological data to support a role for estrogens and ERβ in the suppression of prostatic malignancy, but the details including the exact mechanism of this action remain unproven. ERβ expression is variable; normally expressed at high levels in prostatic epithelium, a number of studies have reported that ERβ expression is lost within PCa cells (Horvath et al. 2001, Leav et al. 2001, Pasquali et al. 2001, Fixemer et al. 2003, Bardin et al. 2004). More specifically, ERβ expression in normal human prostatic epithelial basal cells was reported to be lost in high grade dysplasia, to reappear in Gleason grade 3 cancers, be reduced or lost in more aggressive and higher grade (Gleason grade 4/5) cancer cells but reappear in metastases with a large variability in immunoreactive cells (Leav et al. 2001, Lai et al. 2004). This variability has been attributed to promoter methylation and has been shown to be reversible and stage specific (Zhu et al. 2004). Despite this, the variability of ERβ expression makes it difficult to predict a unifying hypothesis and role for ERβ at different stages of disease.

Additionally, studies on TRAMP mice, which rapidly develop high grade PCa, has shown that the consumption of genistein significantly delays PCa progression (Mentor-Marcel et al. 2001, 2005), and also that this naturally occurring ERβ-specific compound may be a chemopreventative in these mice (Wang et al. 2007). Similar studies in humans have also reported reduced growth or reduced grade of PCa following consumption of dietary supplements containing high levels of phytoestrogens (Landstrom et al. 1998, Onozawa et al. 1998, Bylund et al. 2000, Jarred et al. 2002). Consequently, there is considerable evidence that activation of ERβ has beneficial actions and would appear to have anticarcinogenic properties.

Summary/conclusions

Androgens and estrogens exert similar, yet different, effects in the prostate, and it is becoming clear that a finely tuned balance between the effects mediated by AR, ERα, and ERβ is required for the maintenance of prostate health.

The action of estrogens is complex, having both adverse and beneficial roles via ERα and ERβ respectively. The adverse effects, specifically aberrant proliferation, inflammation, and malignancy all require the presence and activity of ERα and establishes a rationale for the use of ERα-specific antagonists in the chemoprevention of PCa (Ellem & Risbridger 2007). ERβ, however, appears to mediate beneficial effects through preventing hyperplasia and potentially preventing inflammation and carcinogenesis, thus establishing the rationale for using ERβ agonists for the treatment of benign prostatic hyperplasia and, potentially, PCa (Fig. 2; Ellem & Risbridger 2007).

Although the beneficial effects of modulating estrogen receptor activity as a target for treatment of prostate diseases are apparent, the translation of this information into potential therapeutic applications, particularly for PCa, is likely to be highly challenging. This challenge is demonstrated by a number of factors. ERβ expression varies over the course of prostate carcinogenesis, thus limiting the windows of opportunity for use of ERβ-specific compounds. In addition, little is known about the expression or function of receptor splice variants, mutations, ligand-dependent and ligand-independent activities and the role of genomic versus non-genomic signaling (Levin 2001, Matthews & Gustafsson 2003, Bjornstrom & Sjoberg 2005, Wang et al. 2006). Despite this complexity, there is significant potential for the use of targeted estrogen receptor therapies in the prostate and this clearly warrants further investigation.

Acknowledgment

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 11 July 2007

Accepted 20 July 2007

Made available online as an Accepted Preprint 26 July 2007