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Oestrogens and prostate cancer

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Abstract

Androgens are essential for stimulating normal development, growth and secretory activities of the prostate whereas oestrogens are generally regarded as inhibitors of growth. Evidence for the local synthesis of oestrogens includes the detection of aromatase mRNA and protein in the stroma of human non-malignant tissues and in malignant tissue, where it is detected in epithelial tumour cells. As well, aromatase activity was measured by biochemical assay and protein was detected in prostatic non-malignant and tumour cell lines. Taken together with the identification of direct oestrogenic actions on the prostate, these results suggest that alterations in local oestrogen synthesis may have significant consequences in malignancy of these organs.

Genetically modified mouse models were studied in order to evaluate the action of oestrogens alone or in combination with androgens on the prostate gland. Hypogonadal (*hpg*) mice are deficient in gonadotrophins and androgens but showed direct proliferative responses to oestradiol. The responses were characterised by discrete lobe-specific changes including smooth-muscle regression, fibroblast proliferation, inflammation, and basal epithelial cell proliferation and metaplasia. The aromatase knockout (ArKO) mouse, deficient in oestrogens due to a non-functional aromatase enzyme, developed prostatic hyperplasia during the lifelong exposure to elevated androgens, however, no malignant changes were detected in the prostate at any time. In contrast, combined androgen and oestrogen treatment has been shown to induce prostatic dysplasia and adenocarcinoma. These results demonstrate that malignant changes to the prostate gland are dependent upon both androgenic and oestrogenic responses and that neither hormone alone is sufficient to evoke aberrant patterns of growth, resulting in malignancy.

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Introduction

Men and women synthesise both androgens and oestrogens, but the relative ratio of the two hormones between the two sexes is markedly different. The importance of androgens to the male is unequivocal whereas the roles of oestrogens are less clear. Oestrogen synthesis occurs via aromatisation of androgens utilising the aromatase enzyme (cytochrome P450_{arom}); therefore the aromatase enzyme is a critical regulator of the balance between the androgens and oestrogens that contributes to circulating and tissue levels of these hormones. In men, the balance between systemic levels of androgens and oestrogens is altered significantly upon aging; plasma androgen levels decline whereas oestradiol levels remain relatively constant (Vermeulen *et al.* 2002).

In specific tissues of the body, the balance between androgens and oestrogens may differ significantly from that in the

plasma but is nevertheless dependent upon the presence and activity of locally produced steroid metabolising enzymes, such as 5 α -reductase and aromatase (Voigt & Bartsch 1986, Negri-Cesi *et al.* 1998, 1999, Weber *et al.* 1999, Steers 2001). The role of local synthesis of steroids has assumed increasing importance in some disease states, particularly in glandular tissue such as the breast, wherein abnormal levels of oestradiol promote the development and proliferation in the early stages of malignant transformation of epithelial cells (Simpson *et al.* 1994, Santen *et al.* 1997).

Aromatase in prostate

Aberrant expression of aromatase is believed to contribute to the development and progression of breast cancer (Simpson *et al.* 1994, Santen *et al.* 1997). As there is increasing evidence that the prostate is a target for direct oestrogenic

activity (Jarred *et al.* 2000, Prins *et al.* 2001, Putz *et al.* 2001), it is important to determine whether or not aromatase is expressed locally and to identify any changes that may occur with prostate disease. To date, aromatase expression in the prostate is contentious because the detection of enzymic activity or gene expression is equivocal; numerous reports have detected or failed to detect aromatase in prostatic tissue (Smith *et al.* 1982, Kaburagi *et al.* 1987, Stone *et al.* 1987, Brodie *et al.* 1989, Matzkin & Soloway 1992, Tsugaya *et al.* 1996, Hiramatsu *et al.* 1997, Negri-Cesi *et al.* 1998, 1999). We believe that the discrepancies in the literature are partly due to the heterogeneity of prostatic tissue. Aromatase gene expression was detected in human prostate specimens and tumour cell lines using laser capture microdissection (LCM) and RT-PCR. Aromatase protein was also detected by Western blot and enzymic activity was measured in non-malignant and prostate tumour cell lines by tritiated water release assay (Fig. 1). These data provide compelling evidence for the local synthesis of oestrogen in the prostate gland.

Comparison with breast cancer

In the non-malignant breast, aromatase is expressed in the pre-adipocytes, whereas in malignant tissues the epithelial tumour cells also express aromatase (Santen *et al.* 1997). The highest levels of aromatase expression occur in the breast quadrant containing the tumour; the elevated local oestrogen levels in the quadrant with the tumour form a positive feedback loop that drives tumour cell proliferation (O'Neill *et al.* 1988, Bulun *et al.* 1993, Zhao *et al.* 1996, Simpson *et al.*

1997). With the aid of LCM, the cellular site of aromatase expression in human prostate was compared with that reported in the breast. In the prostate, non-malignant stromal tissue expressed aromatase, while benign epithelial cells did not. In contrast to benign tissue, aromatase was also detected in samples of malignant epithelia as well as human prostate tumour cell lines, indicating an induction of gene expression with the onset and/or progression of malignancy. Furthermore, the levels of activity measured in prostate tumour cell lines by tritiated water assay were within, but at the lower end of, the range of activity reported to be present in breast tumours (7.5 fmol/mg protein/h–7.8 pmol/mg protein/h (Dikkeschei *et al.* 1996, Sourdain *et al.* 1996, Brodie *et al.* 1997). Thus aromatase expression and activity in prostate and breast is up-regulated at the tumour site in both tissues.

In breast cancer the importance of increased local oestrogen synthesis via alteration in aromatase enzyme activity at the tumour site relates to the proliferative actions of oestrogen on tumour cells (Simpson *et al.* 1994, Zhao *et al.* 1996). In the prostate gland the increased levels of aromatase enzyme expression and activity in tumour tissues and cells may be equally important, and this raises the question of the role of local, direct responses to oestrogen within the prostate gland itself.

Direct response of the prostate to oestrogens alone

In order to identify the local tissue response to oestrogen we previously utilised *in vitro* organ culture (Jarred *et al.* 2000).

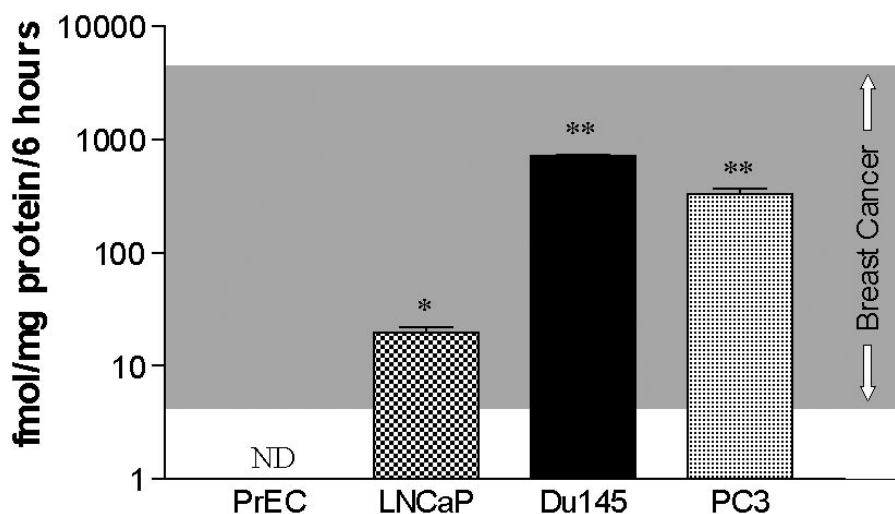


Figure 1 Aromatase activity in human prostate cell lines as measured by tritiated water release assay. The grey area represents the upper and lower activity levels reported in breast cancer (7.5–7800 fmol/mg protein/6 h). PrEC, benign prostate epithelial cell line; LNCaP, androgen-responsive prostate tumour cell line; Du145 and PC3, androgen-independent prostate tumour cell lines. ND, not detectable. * $P < 0.05$, ** $P < 0.01$: statistical significance as determined by *t*-test.

More recently, the direct actions of oestrogens *in vivo* were evaluated using the hypogonadal (*hpg*) mouse model (Cattanach *et al.* 1977) that is deficient in pituitary gonadotrophin and sex steroid production.

Mature male *hpg* mice were exposed to oestradiol for 6 weeks, and proliferative changes were recorded in the prostate with specific effects observed in the stroma and epithelium (Fig. 2); oestradiol administration stimulated growth and expansion of the stromal, epithelial, and luminal compartments of the mouse prostate lobes (Bianco *et al.* 2002). The epithelial cells became multi-layered and squamous (Fig. 2C), showing immunorexpression of high molecular weight cytokeratins and cytokeratin 10 that is characteristic of the pathology known as squamous metaplasia (Cunha *et al.* 2001, Risbridger *et al.* 2001a,b). The stromal response was characterised by an increase in fibroblastic stroma that pen-

etrated the smooth muscle layer, accompanied by a reduction and disorganisation in α -actin-positive smooth muscle cells surrounding the glandular ducts (Fig. 2D). Additionally, neutrophils were identified in the stroma and were shown to migrate through the epithelium to the lumen (Fig. 2C). Although secretory activity was significantly reduced by oestradiol, the lumen was distended as a result of accumulated cellular debris comprising epithelial cells, inflammatory cells, and anuclear keratinised deposits (Bianco *et al.* 2002). Interestingly, this tissue pathology bore a marked similarity to that observed in the β ERKO mouse (Prins *et al.* 2002) and suggests there may be a role for oestrogen receptor β (ER β) in regulating the immune response in the prostate. Overall, oestradiol induced direct proliferative changes in the prostate of the *hpg* mouse, yet these tissues showed no evidence of malignancy.

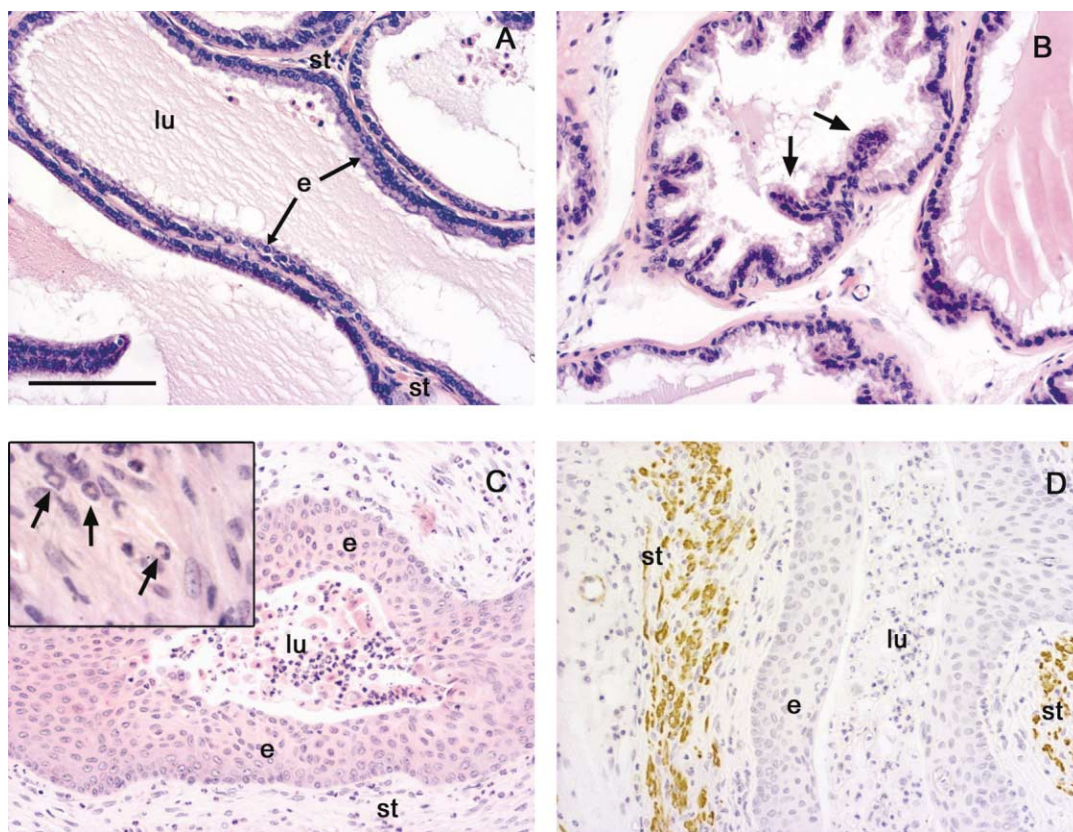


Figure 2 *In vivo* response of prostate to steroid hormones. The prostate of wild-type mice (A) shows secretory and basal epithelial cells (e) lining the prostatic lumen (lu), with the functional ducts surrounded by intervening stroma tissue (st). (B) ArKO mice prostates show benign hyperplasia, characterised by stromal and epithelial cell proliferation and the appearance of epithelial papillary infolding (arrows) as a result of lifelong exposure to elevated androgen. The administration of oestradiol to gonadotrophin-deficient *hpg* mice (C, D) results in squamous metaplasia in the epithelium (e), accompanied by local inflammation characterised by neutrophil infiltration (C, inset, arrows) in the stroma (st), epithelium (e) and lumen (lu). Oestradiol treatment also resulted in the accumulation of cellular debris, inflammatory cells and anuclear keratinised deposits in the lumen. Oestradiol treatment also resulted in stromal fibroblastic proliferation and the disorganisation of the smooth muscle cell layer (D, brown staining). All images show prostates from ~12-week-old male mice. Bar = 100 μ m

Effects of oestrogen deficiency i.e. unopposed androgen action on the prostate

The converse effect of removing oestrogen, both peripherally and locally, was studied in the oestrogen-deficient aromatase knockout (ArKO) mouse. In the absence of oestrogen there is a lifelong elevation of androgens in male ArKO mice, benign prostatic hyperplasia (BPH; Fig. 2B) develops in maturity (McPherson *et al.* 2001). Serum testosterone levels were increased up to eightfold in the ArKO mice and dihydrotestosterone (DHT) levels were also elevated. In the prostate tissue itself the hormone profiles were different: DHT rather than testosterone levels were significantly elevated and were accompanied by an up-regulation in androgen receptor immunoexpression (McPherson *et al.* 2001). As well, serum prolactin was elevated in these mice and may contribute to the emergence of the prostatic phenotype since over-expression of prolactin causes prostatic enlargement (Wennbo *et al.* 1997). Interestingly the ArKO mouse prostate is significantly enlarged as early as postnatal day 3. Using a new method to analyse the pattern of branching morphogenesis of the prostate, increases in the number of branchpoints, branches, branch length and the number of terminal tips were recorded at this early age (G Almahbobi and G Risbridger, personal communication). Further examination of the hormonal profile of these mice is required to resolve whether the phenotype observed in the ArKO prostate is also related to a change in serum prolactin. Nevertheless, BPH occurs in the absence of oestrogen and although there is a lifelong elevation of androgens, no malignant changes are observed.

Conclusions

The evidence presented in this review demonstrates that the human prostate gland may synthesise oestrogens via the local expression of the aromatase enzyme. In malignancy there are changes in the cellular site of aromatase expression, and it is postulated that these tissue-level changes are important in the emergence of, or progression to, prostate cancer in a manner similar to that described in breast cancer.

In vivo, the direct response of prostatic tissues to oestrogen in the *hpg* mouse includes proliferative actions on the epithelia and stroma, as well as the initiation of an inflammatory response. Conversely, oestrogen deficiency in the ArKO mouse causes prostatic enlargement and benign prostatic hyperplasia. Therefore, although oestrogens and androgens are each separately capable of altering the normal growth of the prostate, individually they do not induce prostatic malignancy.

However, it has been shown that, in combination, androgens and oestrogens can induce dysplasia, premalignant and malignant changes to the cells of the prostate as previously reported by several groups (Leav *et al.* 1988, Ho *et al.* 1995,

Wang & Wong 1998, Wang *et al.* 2000, 2001, Hayward *et al.* 2001). As neither hormone by itself is capable of inducing malignant changes in the prostate, the balance between the hormones is critical, not only during normal function, but also in disease. Although systemic hormone levels are important in prostate cancer, a better understanding of hormonal changes within the organ itself is required to demonstrate the significance of changes in the local synthesis and action of oestrogens, in combination with androgens.

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