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Aromatase and regulating the estrogen:androgen ratio in the prostate gland☆

Stuart J. Ellem*, Gail P. Risbridger

Centre for Urological Research, Monash Institute of Medical Research, Monash University, 27-31 Wright Street, Clayton, Victoria 3168, Australia

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ABSTRACT

Although androgens and estrogens both play significant roles in the prostate, it is their combined action – and specifically their balance – that is critically important in maintaining prostate health and tissue homeostasis in adulthood. In men, serum testosterone levels drop by about 35% between the ages of 21 and 85 while estradiol levels remain constant or increase. This changing androgen:estrogen (T:E) ratio has been implicated in the development of benign and malignant prostate disease.

The production of estrogens from androgens is mediated by the aromatase enzyme, the aberrant expression of which plays a critical role in the development of malignancy in a number of tissues. The normal prostate expresses aromatase within the stroma, while there is an induction of epithelial expression in malignancy with altered promoter utilisation. This may ultimately lead to an altered T:E ratio that is associated with the development of disease.

The role of estrogen and the T:E balance in the prostate is further complicated by the differential actions of both estrogen receptors, α and β. Stimulation of ERα leads to aberrant proliferation, inflammation and pre-malignant pathology; whereas activation of ERβ appears to have beneficial effects regarding cellular proliferation and a putative protective role against carcinogenesis.

Overall, these data reveal that homeostasis in the normal prostate involves a finely tuned balance between androgens and estrogens. This has identified estrogen, in addition to androgens, as integral to maintaining normal prostate health, but also as an important mediator of prostate disease.

1. Introduction

Benign and malignant disorders of the prostate are amongst the most common diseases affecting males, particularly in industrialized countries. Recent statistics demonstrate that prostate cancer (PCa) is now the most commonly diagnosed cancer in men in developed countries, with the highest incidence in North America followed by Australia/New Zealand [1]. With demographic changes indicating further aging of the population, this prevalence of prostate disease will continue to rise and represents an increasingly significant burden for modern healthcare.

Androgens have been the traditional focus of prostate research and there is universal agreement that androgens play a critical role in normal prostatic growth, development, and the maintenance of tissue homeostasis. Additionally, androgens also play a central role in the development of prostate disease. Although testicular testosterone (T) provides the main source of androgens in the prostate, the gland also has the capacity to locally metabolise the more potent androgen, dihydrotestosterone (DHT), via reduction of testosterone by the 5α-reductase enzyme. Given the central role that androgens play in prostate development and disease, prostatic androgen metabolism has been vigorously investigated, and, consequently, androgen ablation therapy has been the mainstay of treatment for PCa ever since the pioneering works of Huggins and Hodges more than 60 years ago [2,3].

However, despite the well-documented importance of androgens, our understanding of the pathophysiological processes involved in prostate disease remains incomplete. In particular, androgens may also be metabolised to estrogens (E) via the action of the aromatase enzyme and there is a growing body of evidence that implicates estrogens in the aetiology of prostate disease. Significantly, the aberrant expression of aromatase has been implicated in the disease process in other tissues such as the breast and endometrium [4–12]. The role of estrogens in the prostate and the prostatic disease process has received less attention than that of androgens, despite emerging recognition that estrogens – in addition to androgens – play an essential role.

The development of genetically modified mice that have altered aromatase expression, the aromatase knock-out (ArKO) and the aromatase over-expressing (AROM+) mice, provide unique tools to examine the importance of estrogen and the testosterone:estrogen (T:E) ratio in the prostate. Collectively, this work has revealed that elevated testosterone in the absence of estrogen leads to the development of hypertrophy and hyperplasia, but not malignancy. In contrast, high estrogen and low testosterone has been shown to lead to the development of inflammation upon aging and the

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* Corresponding author. Tel.: +61 3 9594 7408; fax: +61 3 9594 7420.
E-mail address: Stuart.Ellem@med.monash.edu.au (S.J. Ellem).

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emergence of pre-malignant lesions. The role of estrogen and the T:E balance in the prostate is further complicated as the specific effect of estrogen is also dictated by the differential actions of both estrogen receptors, ERα and ERβ. Specifically, it is the activation of ERα that leads to aberrant proliferation, inflammation and the development of pre-malignant lesions, whilst, in contrast, the activation of ERβ mediates anti-proliferative, anti-inflammatory and, potentially, anti-carcinogenic effects that balance the actions of ERα as well as those of androgens.

This review considers the role of aromatase and estrogens in the prostate and in prostate disease, specifically in relation to the T:E balance and the differential actions of estrogen via each estrogen receptor subtype, ERα and ERβ. Overall, we conclude that estrogens, in addition to androgens, are essential for normal prostate growth and development but also play a role in the development of prostate disease. This role is specifically mediated and influenced by the local actions of aromatase, the T:E ratio and the expression and action of each of the estrogen receptors.

2. Estrogens and the prostate

Although the incidence of PCa increases with age and is dependent upon androgens, the development of PCa commonly occurs at an age in men when serum testosterone levels are in decline [13,14]. Testicular testosterone synthesis and serum testosterone levels fall as men become older, but the levels of estradiol do not; remaining unchanged or increasing with age [13,15–19]. Consequently, there is a significant change to the T:E ratio that is temporally associated with the onset of prostate disease, including PCa.

The role and importance of androgens in the development of PCa is well known, however, we now know that androgens alone are insufficient to induce tumourigenesis [20]. Specifically, when given estrogens in conjunction with androgens, the Noble rat develops pre-cancerous lesions and prostate adenocarcinoma [21–23]. The combined T and E treatment of grafted prostatic tissues also promotes malignancy in Rb-deficient mouse tissue recombinants [24] and, similarly, the treatment of wild-type mice with high doses of both hormones induces prostatic hyperplasia, dysplasia and carcinoma *in situ* [25]. This reinforces the importance of the T:E ratio, and, in particular, demonstrates the importance of estrogens in addition to androgens in the development of malignancy.

Epidemiological evidence provides further support for the relationship between a shift in the T:E ratio and the development of PCa. This ratio is significantly lower in African-American men (who have the highest incidence of PCa in the USA), due to higher levels of serum estrogens, compared to Caucasian-American men [23,26–28]. Conversely, the T:E ratio is higher in Japanese men (who are known to have a low risk of PCa), due to lower levels of serum estrogens, compared to Caucasian-Dutch men [29].

Estrogens exert both adverse and beneficial effects within the prostate and, consequently, it becomes important to consider the source of estrogen. Although estrogens may be introduced to the body exogenously via the diet, the imbalance between androgens and estrogens, and the shift in the T:E ratio, in older men occurs specifically as a result of endogenous changes to steroid metabolism. Alterations to hormone metabolism have been demonstrated at sites distant to the prostate gland itself and of the total circulating estrogen in younger males, the majority (75–90%) is produced in the peripheral tissues through the actions of the aromatase enzyme [30].

In addition to changes in systemic hormones, it is important to consider the local intra-prostatic conversion of androgens to reduced androgens and estrogens, particularly as intra-prostatic androgen levels do not always mirror systemic levels [31] and are critical determinants of prostate health. The levels of intra-prostatic hormones are dependent upon the presence and activity of local steroid metabolising enzymes. Testicular androgens reaching the prostate gland are predominantly converted to DHT by 5α-reductase but may also be converted to estrogen via the action of the aromatase enzyme.

3. Aromatase and the prostate

Estrogens exert significant effects upon the prostate and, in addition to androgens, also play a pivotal role in the development of PCa. Furthermore, the T:E ratio is also an important factor in the initiation of development of PCa. Consequently, it is imperative to consider local prostatic aromatase expression and its potential role in PCa, particularly with regards to its potential influence upon the local intra-prostatic T:E balance.

Previous evidence of aromatase expression in the prostate was equivocal and controversial. A number of studies successfully demonstrated aromatase expression in the prostate by RT-PCR as well as its enzymatic activity by biochemical assay [32–37]. In contrast, other studies failed to demonstrate the presence or activity of aromatase [38–41]. Similarly, work examining the utilisation of the different aromatase promoters in prostate tissues also resulted in contradictory results [36,41].

More recent studies from our laboratory utilising laser capture microdissection (LCM) demonstrated that aromatase is expressed in the benign human prostate [42]. Furthermore, we were also able to show that it was the non-malignant stromal tissue that expressed aromatase and was driven via the PII promoter, while benign epithelial cells do not express aromatase. In light of these data, the normal and benign prostate clearly has the capacity to locally metabolise androgens to estrogens via aromatase. Since estrogens are also implicated in PCa, the question then arises as to how aromatase gene expression might contribute to the aetiology and/or progression of malignancy.

The aberrant expression of aromatase plays a significant role in the development of estrogen dependant neoplasms, most notably the breast where it has been extensively studied [4–10] and to a lesser extent in endometrial cancer [11,12]. Given the structural and developmental similarities existing between breast cancer and PCa [43] and the fact that aromatase is expressed in the prostatic stroma, it is also important to understand whether altered aromatase expression may be involved in the development of PCa.

Much like the data examining aromatase in benign tissue, aromatase expression and activity in PCa and the PCa cell lines had been contentious for some time [32–41]. However, we demonstrated altered aromatase expression in PCa [42]. In contrast to the benign tissue, we found that aromatase was expressed in the epithelium of samples of malignant tissue prepared by LCM, as well as in the human prostate tumour cell lines, indicating an induction of gene expression with the onset and/or progression of malignancy. Analysis of the promoters used in the malignant tissue demonstrated utilisation of PII as well as promoters I.3 and I.4 in LCM and LNCaP samples. In contrast, only I.3 and I.4 transcripts were detectable in DU145 and PC3 cells. These data demonstrate that there is an induction of aromatase expression in the prostate tumour cells with altered promoter utilisation, in a manner analogous to that in the breast and in breast cancer. Furthermore, when aromatase activity was measured by tritiated water release assay in the cell lines, the lowest activity was seen in LNCaP cells with the highest in PC3 cells [42]. Significantly, the levels of activity measured in the prostate cell lines 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 to 7.8 pmol/mg protein/h) [4,10,44].

These combined data suggest that aromatase expression and activity in prostate may be up-regulated at the tumour site, ultimately resulting in an altered local hormonal milieu and T:E ratio. In breast cancer the importance of increased local estrogen

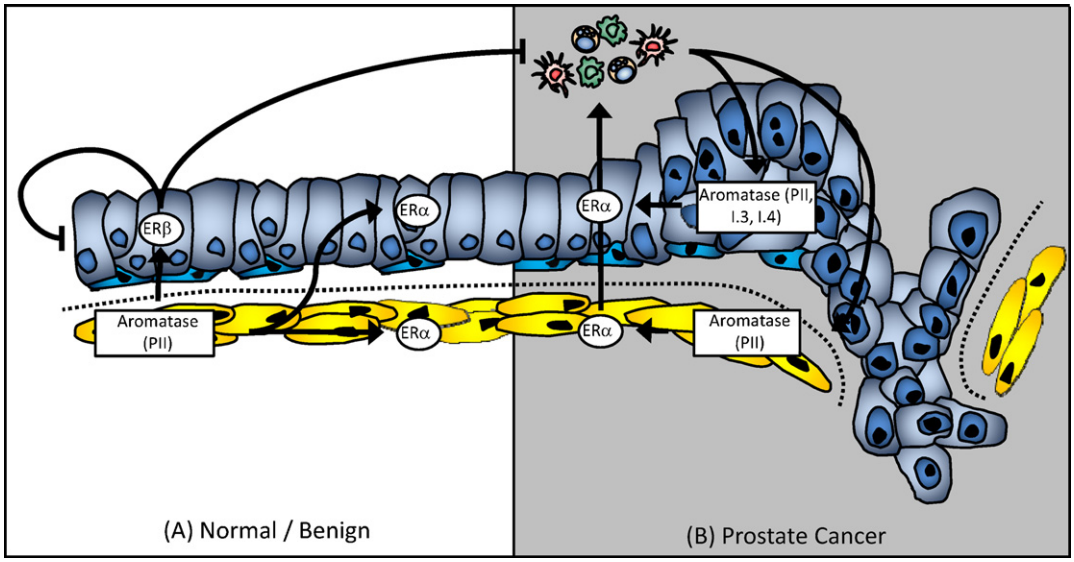

Fig. 1. Effects of aromatase and estrogen signalling in the prostate. (A) In the normal and benign tissue, aromatase is expressed exclusively within the stroma and is regulated via promoter PII. Estrogen then exerts its effects in an autocrine fashion via the stromal ERα, but also in a paracrine manner via the epithelial ERα and ERβ. In this situation the proliferative and pro-inflammatory actions of ERα are balanced by the anti-proliferative and anti-inflammatory actions of ERβ. (B) In prostate cancer, aromatase is now expressed within the epithelial tumour cells, as well as in the stromal cells, and is aberrantly regulated by promoters I.3 and I.4 in addition to promoter PII. Consequently, estrogen now exerts its effects in an autocrine manner via stromal and epithelial ERα and ERβ. The resultant increased levels of estrogen and aberrant ERα signalling promote the development of inflammation, which also drives further increased aromatase expression and may potentially result in the development of a positive feedback cycle (with inflammation driving aromatase expression, thus increasing estrogen levels, which, in turn, promotes further inflammation).

synthesis via altered aromatase enzyme activity at the tumour site relates to the proliferative actions of estrogen on tumour cells [45,46]. In PCa, the increased levels of aromatase enzyme expression and activity in tumour tissues and cells may be equally important as estrogen receptors within the prostatic epithelium are responsive to estrogen in an autocrine fashion, in addition to paracrine signalling from the stroma (Fig. 1).

It has also been postulated that a positive feedback loop, established via paracrine and autocrine mechanisms, leads to the continuing growth and development of breast tumours [47]. Similar processes are likely to be highly relevant in the development and progression of hormone dependent neoplasms in other tissues, particularly the prostate. Significantly, the aromatase promoters utilised in the benign prostate and in PCa are responsive to, and may be driven by, inflammatory cytokines. Estrogens are also capable of inducing prostatic inflammation [48], and, consequently, this is likely to serve to establish a cycle of increased aromatase, local estrogen metabolism and subsequently greater inflammation. This putative mechanism is of particular interest as inflammation has also been implicated in the development of PCa [49–51] and may be a mechanism through which estrogen induces the development of malignancy in the prostate (Fig. 1).

4. ERα and ERβ in the prostate

Estrogens play a myriad of roles in the prostate and the specific effects of estrogen locally within the prostatic tissue are mediated by ERα and ERβ. The effects that are mediated by each of these receptors are quite different and appear to act in opposition. Overall, the prostatic response to estrogen being dictated not only by the T:E ratio, as discussed earlier, but also by the local balance between, and the actions of, these two receptors.

4.1. ERα

The role of ERα in the prostate is one that has been extensively studied by various groups over the years. What has emerged from these studies is that ERα appears to mediate adverse rather than beneficial effects in the prostate. Specifically, estrogen acting via ERα is capable of inducing three different and distinct pathologies in the prostate: aberrant proliferation, inflammation and PCa.

Estrogens are capable of causing proliferation of the prostatic epithelium; however, this effect is distinct to that regulated by androgens. The proliferative response to estrogens is characterised by proliferation in the basal layer of the epithelium and is termed squamous metaplasia (SQM) [52–54]. This proliferative response to estrogen is observed in βERKO but not αERKO mice, and by using tissue recombination techniques, we previously demonstrated that both stromal and epithelial ERα expression is required for the induction of SQM by estrogen [55].

Estrogens are also capable of inducing inflammation in the prostate, as apparent from the pharmacological administration of estrogen to rodents as well as high dose estrogen therapy given to transsexual males. These data are supported by our more recent observations examining the AROM+ mice, which demonstrate extensive inflammation in the prostate tissues upon aging [56]. The observation of inflammation in the prostate of aged AROM+ animals is significant and is comparable to that reported in mice transiently treated with estrogens during neonatal life [57–59]. This inflammation results from the exposure to estrogens and requires the ERα subtype for its mechanism of action, as was shown by Prins and colleagues, where estrogenised wild-type and βERKO mice developed inflammation upon aging but αERKO mice did not [60]. Thus, ERα is the dominant ER subtype mediating the inflammatory response to estrogen of the prostate gland.

A role for estrogen has also been implicated in the development of PCa, as discussed earlier. This induction of malignancy in response to estrogen also hinges upon the pivotal and essential role of ERα. Studies using the αERKO and βERKO mice by Ricke and colleagues have demonstrated that estrogen induced dysplastic changes and the subsequent development of pre-malignancy is mediated specifically by ERα, and not ERβ, as this aberrant response is specifically attenuated in the absence of ERα [61].

This role of estrogens, and particularly ERα, in PCa is also supported by the progressive emergence of ERα expression specifically within the tumour itself [62]. ERα expression emerges within high

grade tumours, but is particularly apparent in hormone refractory tumours and metastases [62]. This emergent ERα expression also correlates with increased progesterone receptor expression [63]. As the progesterone receptor is a well-established marker for ERα activity [64], its increased expression clearly demonstrates an increasing activity of a functional ERα signalling pathway with tumour progression.

4.2. ERβ

In addition to the adverse effects of estrogen in the prostate that are facilitated by ERα, there is substantial evidence to suggest that estrogens also have important, essential and beneficial effects in the prostate. These effects are not mediated by ERα, but, rather, by ERβ. Specifically, these data indicate that ERβ mediates beneficial anti-proliferative, anti-inflammatory, and, potentially, anti-carcinogenic effects of estrogen.

ERβ was only identified and described relatively recently [65] and the specific roles of ERβ within the prostate have just begun to emerge. Coupled with epidemiology and the identification of preferential binding of isoflavones to ERβ [66–69], the available data indicates that ERβ may be integral in regulating proliferation within the prostatic tissue.

An anti-proliferative role for ERβ within the prostate is supported by studies in the ArKO mouse which, in the absence of estrogen develops prostatic hypertrophy and hyperplasia [20]. In addition, we were able to definitively demonstrate that the specific stimulation of ERβ ablates this hyperplastic epithelial cell proliferation and prevents the onset of aberrant hyperplastic growth within the prostatic epithelium [70,71]. This anti-proliferative role of ERβ also concurs with earlier reports that the adult βERKO mouse develops aberrant proliferative lesions within the prostatic epithelium and has proliferative activity more than three times greater than that in normal mice [72,73]. Although there is some doubt surrounding these data, this putative onset of aberrant proliferation in the absence of ERβ activation would be consistent with the anti-proliferative role proposed for ERβ in the prostate.

It has also been shown that estrogens, acting via ERα, are capable of inducing inflammation. However, and in keeping with the opposing actions of ERα and ERβ, other data indicate that ERβ mediates anti-inflammatory effects. A number of animal models of disease such as bladder cystitis, inflammatory bowel disease and microglia have reported possible beneficial effects of ERβ specific agonists on inflammation specifically by inhibiting NFκB transcriptional activity [74,75]. Additionally, studies using the βERKO mouse have also indicated that ERβ may also play an immunomodulatory role in the prostate. Specifically, these mice were found to develop significant and chronic inflammation whilst their wild-type litter mate controls did not [76]. In addition, the activation of ERβ with a specific agonist has also been shown to be able to prevent the development of prostatic inflammation in luteinizing hormone receptor knock-out mice [77].

A number of studies conducted on human PCa tissues have proposed a link between a loss of ERβ expression and the onset or progression of PCa [62,78–83]. Additionally, evidence of hypermethylation of the ERβ gene has also been reported in PCa [84,85]. Further anecdotal epidemiological studies have also suggested that the stimulation of ERβ may have beneficial effects in the prevention of prostate diseases, such as PCa; however, the mechanisms behind these actions remain poorly understood [82,86–90].

5. Conclusions

The hormone balance and ratio of androgens to estrogens plays a pivotal role in prostate disease, particularly during late life. Significantly, the intra-prostatic hormone levels do not always mirror systemic levels and, ultimately, it is the local hormone levels that are important for the maintenance of prostatic health as well as in the development and progression of prostate disease.

Aromatase is expressed locally within the prostate and is aberrantly expressed in PCa (Fig. 1). Specifically, the induction of expression and altered promoter utilisation with malignancy implies a shift in the local hormone balance and T:E ratio. This balance of androgens and estrogens is critical for prostate health, and, consequently, any alteration in aromatase expression has the potential to shift this balance and exert profound effects via ERα, ERβ and/or non-receptor mediated effects.

Estrogen has been implicated in the aetiology of prostate cancer, although the specific mechanisms underlying this role have yet to be elucidated. The observation of inflammation in the prostate of aged AROM+ animals is of particular significance as inflammation has been implicated in the development of PCa. Consequently, estrogen induced inflammation may be a mechanism promoting the development of malignancy in response to estrogen. Furthermore, given the potential of pro-inflammatory cytokines to drive aromatase expression, a positive feedback loop between estrogen production and inflammation may result, driving the development and progression of PCa (Fig. 1).

Whilst aromatase is clearly an important factor determining the T:E ratio in the prostate and in prostate disease. It is, however, important to recognise that other steroidogenic enzymes may also affect this ratio, particularly in PCa. Steroid sulfatase (STS) and estrogen sulfotransferase (EST), responsible for the conversion of estrogen-sulfate to and from estrone, are both reported to have increased expression in PCa [91]. Significantly, both STS and EST, along with aromatase, play a significant role in other hormone dependant cancers, such as breast cancer. The 17β hydroxysteroid dehydrogenases, 17β-HSD-1 and 17β-HSD-2, are involved in the conversion between estrone and estradiol and are also expressed in the prostate. 17β-HSD-1 was expressed but unchanged in PCa [91,92], while the expression of 17β-HSD-2 was decreased [92]. This would suggest a predominant conversion of estrone to the more potent estradiol in PCa. The combined effect of these enzymes will have a significant effect upon local estrogen levels and the intra-prostatic T:E ratio. As a result, they may play important roles in the development and progression of PCa, along with aromatase.

Overall, estrogen and androgen hormone action in the prostate remains an important area for investigation. There is now a significant body of evidence supporting the role for estrogens, in addition to androgens, in the aetiology of PCa. It is imperative that we fully understand the nuances of androgen and estrogen metabolism and signalling in the prostate, particularly as it relates to the development and progression of PCa, as this knowledge will be fundamental to the development of new therapies for this disease.

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