REVIEW

Sex steroids in the tumor microenvironment and prostate cancer progression

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Abstract

Prostate cancer is uniquely dependent on androgens. Despite years of research on the relationship between androgens and prostate cancer, many questions remain as to the biological effects of androgens and other sex steroids during prostate cancer progression. This article reviews the clinical and basic research on the influence of sex steroids such as androgens, estrogens and progesterone within the prostate tumor microenvironment on the progression of prostate cancer. We review clinical studies to date evaluating serum sex steroids as prognostic biomarkers and discuss their respective biological effects within the prostate tumor microenvironment. We also review the link between genomic alterations and sex steroid levels within prostate tumors. Finally, we highlight the links between sex steroid levels and the function of the immune system within the tumor microenvironment. As the context of treatment of lethal prostate cancer evolves over time, an understanding of this underlying biology remains central to developing optimal treatment approaches.

Introduction

The significance of androgens and other sex steroids during androgen deprivation therapy (ADT) continues as an area of further exploration over 70 years since Huggins and Hodges first described the androgen dependency of prostate cancer (PCa) (Huggins & Hodges 1941). Androgens, as well as estrogens and progesterone, appear to play a significant role in not only carcinogenesis, but also on the progression of PCa. Castration remains the primary treatment for advanced prostate cancer, with androgen receptor (AR) pathway inhibitors used for castration-resistant prostate cancer (CRPC). Through the development and subsequent treatments for PCa, endocrine and paracrine systems regulate a prostate tumor microenvironment (TME), which is constantly exposed to circulating sex steroids. As our understanding of how circulating and locally produced sex steroids impact cell autonomous and non-cell autonomous mechanisms of tumor resistance within the TME advances, we may better harness the ability of local and circulating sex steroids to act as biomarkers.

Historic interest in the role of sex steroids on PCa progression dates mostly to the role of adrenal androgens and intratumoral androgen synthesis (Bélanger et al. 1984). Combined blockade of adrenal androgens with AR antagonism underwent extensive clinical evaluation, but the net clinical benefit was ultimately marginable (Lukka et al. 2006). Nonetheless, the success of the steroidogenesis inhibitor abiraterone as treatment for CRPC highlights the importance of understanding this biology (de Bono et al. 2011, Ryan et al. 2013a).

More recently, several important discoveries have re-ignited interest as to the importance of sex steroids
Sex steroids and steroidogenesis

Sex steroids are all biochemically based on a 17-carbon 4-ring backbone. Androgens and their metabolites have 19 carbon molecules, estrogens have 18 carbons, progestogens, glucocorticoids and mineralocorticoids have 21 carbons. Clinically, sex steroids are defined by their effects on sexual differentiation and maturation, with effects recognized to be dependent on concentration and exposure. In the cell, sex steroids may bind to cognate receptors, translocate to the nucleus and affect transcriptional changes or alternatively induce changes through non-receptor mechanisms.

Biosynthesis of sex steroids is performed by multiple enzymes through multiple intermediates. A summary and simplification of the major intermediates and key enzymes is presented in Fig. 1. Unique gene transcripts encode each enzyme, with tissue-specific expression throughout the body. In men, androgens are primarily synthesized by the Leydig cells of the testis and in the glomerulosa reticulata of the adrenal gland. Steroidogenesis is highly complex, and there remains much detail, which is incompletely understood. A summary of the function of steroidogenesis enzymes is reviewed in detail elsewhere (Zhang et al. 2016).

The hormonal milieu within prostate tumors is influenced by both intratumoral production of sex steroids as well as the circulating endocrine system. Levels of sex steroids in the circulation are carefully regulated by the hypothalamic pituitary axis as well as via the adrenal cortex. Obesity is another key regulator of systemic sex steroid levels, with peripheral conversion of testosterone to estrogens occurring via aromatase in adipocyte tissue. The association of periprostatic adipose tissue with more aggressive PCa may also represent a more local source of estrogenic conversion (Toren & Venkateswaran 2014).

The measurement of sex steroids is a common confounder in many studies and creates difficulties in interpreting the literature; guidelines exist for standardized methodology endorsed by the Endocrine Society. Determination of serum sex steroids is most accurate using LC–MS/MS methods, while immunohistochemical or radioimmunoassay methods are less reliable. Overall, the complexity of the biology as well as the many confounders including measurement accuracy present important potential confounders to consider when evaluating clinical studies measuring sex steroids in PCa patients.

Changes in sex steroids during prostate cancer progression

Physiologic changes in aging men

Prostate cancer occurs predominantly in older men. Several changes in sex steroids have been described in the serum and prostate tissue of older men. In the serum, levels of testosterone and its metabolite dihydrotestosterone (DHT) tend to decrease gradually with age (Araujo et al. 2004, Yeap et al. 2014). Estradiol levels also tend to slowly decrease with time, but not always to the same extent given the moderating effect of increased peripheral aromatization caused by obesity (Vermeulen et al. 2002).

Benign prostatic hyperplasia (BPH) also variably occurs in men as they age. Sex steroid levels in serum and prostate tissue were recently accurately characterized in men without PCa (Neuzillet et al. 2017). Comparing the differences in serum and tissue sex steroids between...
smaller prostates (<50 g) and those affected by BPH (>50 g), the authors found intraprostatic DHT levels correlate with prostate size. Similar results have also been described by others (Olsson et al. 2011, van der Sluis et al. 2012). Comparing ratios of steroids as surrogates of enzymatic activity, there was significant lower in DHT:estrone (E1) ratios in smaller prostates. As seen with prior research (Niu et al. 2011), their results suggest that BPH is related to greater 5α-reductase (5ARI) activity, while smaller prostates may prefer aromatization of testosterone to estrogens. These differences in tissue steroid homeostasis by prostate size are interesting to PCa progression given that smaller prostate size is known to be an independent predictor of high-grade PCa (Newton et al. 2010).

**Relationship between serum and prostate tissue sex steroid levels**

The relationship between circulating and prostate tissue levels of androgens has been addressed in several studies (Bartsch et al. 1986, Miyoshi et al. 2014, Cook et al. 2017, Neuzillet et al. 2017). Overall, intraprostatic levels of DHT and testosterone, as well as dehydroepiandrosterone (DHEA) and androgen metabolites such as androstane-3β,17β-diol (3β-diol) are 5- to 10-fold higher in prostate tissue than those in the serum. Recently, Cook and coworkers analyzed the relationship between serum and prostate tissue sex steroids using matched samples from 251 patients undergoing radical prostatectomy for PCa (Cook et al. 2017). Their analyses demonstrate that serum
levels of sex steroids were generally poor surrogates for prostatic levels. Nonetheless, there was partial correlation between the individual serum and prostate levels, which was the highest for 3β-diol glucuronide, followed in order by testosterone, DHT and estrone. Thus, despite being continuously bathed in circulating steroids, prostate sex steroid levels have low correlation with circulating levels in patients with localized PCa.

Further, randomized trials in healthy men suggest that testosterone administration does not significantly alter prostatic androgen levels in the short to medium-term (Page et al. 2011). For example, Mostaghel and coworkers performed a ten-week study that showed testosterone supplementation induced no change in prostatic androgens compared to placebo, while synthetic estrogen reduced intraprostatic DHT by 40% (Mostaghel et al. 2012). Overall, neither testosterone nor estrogen administration resulted in significant changes in AR-related gene expression in prostate epithelium. Another study in healthy castrated men examined the impact of exogenous testosterone on intraprostatic androgen levels. In this population, they found exogenous testosterone raised serum levels of both testosterone and DHT and raised intraprostatic testosterone levels. However, no significant changes in intraprostatic DHT, the major androgen in the prostate, were obtained with supplementation (Thirumalai et al. 2016). While removal of the prostate has been associated with decreased serum DHT levels (Olsson et al. 2010), overall, these data suggest tissue-specific regulation of sex steroids occurs within the prostatic milieu, which is partially independent of serum levels.

**Sex steroids and prostate cancer carcinogenesis**

It remains an actively investigated question whether serum or tissue sex steroid levels are associated with the development of PCa. Age, ethnicity and obesity play a significant confounding role in understanding this risk. For example, higher body mass index (BMI) is associated with increased serum levels of estrogens as well as larger prostate size (Joseph et al. 2002), while at the same time, estradiol levels, but not testosterone, are higher in African American men compared to Caucasian men (Litman et al. 2006, Rohrmann et al. 2007). The high prevalence of indolent cancer and variable serum PSA thresholds for biopsy are other important confounders. Multiple studies have assessed the role of testosterone levels related to PCa risk. However, the relationship between testosterone or DHT levels, exogenous testosterone and overall PCa risk remains inconsistent in the literature, in part due to low methodological quality (Muller et al. 2012, Klap et al. 2015). The literature does appear to support that lower serum testosterone at diagnosis is associated with more aggressive PCa (Botto et al. 2011, Dai et al. 2012, Garcia-Cruz et al. 2012, Park et al. 2016, Lukić et al. 2017).

Recently, large drops or large fluctuations of a man’s serum testosterone levels in the past were also associated with risk of PCa (Xu et al. 2017). Similarly, low serum DHEA levels were associated with more aggressive localized PCa (Miyoshi et al. 2016). The role of other sex steroids, if any, on PCa prevalence remains unclear. Ecologic studies have raised the question whether estrogens could be related to PCa development, with serum estrone previously linked to PCa risk (Margel & Fleshner 2011, Kristal et al. 2012). Lower estradiol levels in PCa cases vs controls have also been reported (Schatzl et al. 2000). Careful consideration of confounders, as well as accurate measurement techniques will be crucial to understand if there exists any link between serum sex steroid levels and PCa carcinogenesis.

5ARI inhibitors are known to decrease intraprostatic dihydrotestosterone levels while increasing intraprostatic testosterone (Marks et al. 2001, Mostaghel et al. 2012). The Prostate Cancer Prevention Trial (PCPT) and Reduction by Dutasteride of Prostate Cancer Events (REDUCE) clinical trials successfully demonstrated the ability of finasteride and dutasteride, respectively, to decrease by about 25% the detection by biopsy of indolent PCa; yet, both studies showed a concomitant 0.5% absolute increase in high-grade cancer (Thompson et al. 2003, Andriole et al. 2010). Taken together, these studies indicate decreases in prostate tissue androgens by 5ARIs may decrease the prevalence of indolent cancer, but they do not provide sufficient evidence to indicate that changes in serum or prostatic sex steroid levels are related to any increased incidence of high-grade PCa.

**Changes in androgens and estrogens following androgen deprivation therapy**

Luteinizing hormone-related hormone (LHRH) agonists and LHRH antagonists function to decrease serum free and total testosterone, with a nadir value generally obtained within the first year (Xu et al. 2002, Gulley et al. 2005, Nishii et al. 2012). In addition, serum adrenal androgens may decrease to a lesser extent following LHRH antagonists (Miyazawa et al. 2017). Studies with orchietomy or LHRH agonists suggest that DHEA does not change substantially following ADT (Xu et al. 2002, Oka et al. 2003). One study reported that 5-androstenediol
levels do not change following castration (Mizokami et al. 2004). However, in men treated with estrogens, a decline in DHEA levels is observed concomitant with increases in sex hormone-binding globulin levels (Aggarwal et al. 2009). The authors also reported that a decline in serum DHEA-sulfate and a rise in DHT are both associated with a PSA response in men treated with estrogens. Following LHRH agonist therapy, estradiol levels typically decrease by about 90% (Nishii et al. 2012).

In prostate tissue, ADT is accompanied by a decrease in testosterone and DHT levels, though adrenal androgens may not be significantly altered (Arai et al. 2011). Notably, prostatic testosterone and DHT levels decrease less following ADT compared to non-prostate tissues (van der Sluis et al. 2012). Neoadjuvant prostatectomy studies have evaluated prostate tissue androgens following ADT. For example, Mostaghel and coworkers found that while serum androgens drop by ~95% following ADT, intraprostatic androgens decline by only 70% (Mostaghel et al. 2007). A neoadjuvant study comparing an LHRH antagonist and LHRH agonist did not suggest the differences in prostate tissue T or DHT between treatments (Sayyid et al. 2017). Another neoadjuvant prostatectomy study clearly established that combinations of LHRH agonist with CYP17 inhibitors or 5ARIs can further lower prostate tissue androgens decline by only 70% (Montgomery et al. 2008). Another study reports serum testosterone levels increase following 8 weeks of enzalutamide therapy for CRPC (Efstathiou et al. 2015). The increased age of CRPC patients may be a significant confounder in interpreting testosterone levels and may explain why serum testosterone levels were prognostic of survival in men starting abiraterone therapy post chemotherapy (Ryan et al. 2013b).

### Genomic alterations implicating sex steroids in prostate cancer progression

With steroidogenesis implicated in the progression of PCa, multiple studies have assessed the role of single nucleotide polymorphism (SNPs) in genes related to sex steroids on the risk of PCa progression. Differences in serum sex steroids have been reported to vary by ethnicity (Jakobsson et al. 2006, Nadeau et al. 2011, Levesque et al. 2013). It is notable that despite the low prevalence of genetic aberrations in localized prostate cancer, many of these somatic polymorphisms have significant effects on prostate cancer progression and outcome (summarized in Table 1).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Association</th>
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<tr>
<td>SLC02B1</td>
<td>Increased expression in CRPC samples compared to localized PCa samples (Wright et al. 2011)</td>
</tr>
<tr>
<td>SLC01B3</td>
<td>Increased expression in CRPC samples compared to localized PCa samples (Wright et al. 2011)</td>
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<tr>
<td>SLCO1B3</td>
<td>Increased expression in CRPC samples compared to localized PCa samples (Wright et al. 2011)</td>
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Somatic SNPs in androgen transport genes SLC02B1 and SLC01B3 are implicated in altering the duration of response to ADT (Wright et al. 2011, Yang et al. 2011, Wang et al. 2016). SLC02B1 and SLC01B3 are anionic transporters, which facilitate specific steroids to cross the cell membrane (reviewed in Cho et al. 2014). Expression of SLC01B3 and SLC02B1 is higher in metastatic prostate samples compared to localized PCa samples (Wright et al. 2011). SNPs of SLC01B3 result in increased testosterone transport into cells. These SNPs are associated with both sooner time to CRPC as well as overall survival (Hamada et al. 2008, Sharifi et al. 2008, Yang et al. 2011). Similarly, time to castration resistance has been reported lower
in men with SNP variants of \textit{SLCO2B1} (Fujimoto et al. 2013). These \textit{SLCO2B1} variants result in increased DHEA transport into cells. Statins are recognized inhibitors of \textit{SLCO2B1} transport and may explain why a longer time to progression for men treated with a statin at the time of ADT initiation was seen in a large cohort of men receiving ADT (Harshman et al. 2015). This was seen in men with and without metastases after adjusting for prognostic factors.

\textit{SRD5A1} and \textit{SRD5A2} genes encode different isoforms of the \( \alpha \)-reductase abundant in the prostate, which converts testosterone into the more potent DHT. A meta-analysis concluded that the \textit{SRD5A2} rs9282858 polymorphism is associated with increased risk of PCa (Fang et al. 2017). A small Japanese study of 86 patients found that lower serum testosterone levels during ADT were associated with the CC allele in the \textit{SRD5A2} gene (rs523349), and this conferred a better prognosis (Shiota et al. 2016). The same group previously also reported that the rs523349 \textit{SRD5A2} SNP conferred a worse prognosis for men treated with primary ADT (Shiota et al. 2015). Polymorphisms of \textit{SRD5A1} and \textit{SRD5A2} have also been associated with recurrence of PCa post-prostatectomy (Audet-Walsh et al. 2011).

In two large independent cohorts, including men on ADT, Levesque and coworkers found that SNP variants in \textit{CYP17A1} (rs6162), \textit{HSD17B2} (rs4243229 and rs7201637) and \textit{ESR1} (rs1062577) were associated with PCa and overall survival (Levesque et al. 2013). Moreover, these SNPs were associated with altered levels of circulating sex steroids. A variant in the \textit{CYP11A1} gene has been associated in one study with an increased risk of metastatic PCa (Kumazawa et al. 2004).

HSD3B1 is a key enzyme in the steroidogenic synthesis pathway (Fig. 1). Expression of the 1245C SNP results in increased HSD3B1 protein levels via inhibition of ubiquitin-mediated degradation and subsequently a greater flux toward DHT synthesis (Chang et al. 2013). Heterozygous HSD3B1 variant (1245C) allele has been associated with a shorter time to CRPC in a Chinese cohort of 108 patients where the heterozygote prevalence was 17\% (Wu et al. 2015). In a multicohort Caucasian population, the variant allelic frequency was 29\% (Hearn et al. 2016). Men homozygous for 1245C variant in this study had non-significantly higher proportions of high-stage T3b-T4 disease and high-grade Gleason 8–10 disease and in one cohort significantly higher proportions of N1-positive disease. In all cohorts, progression-free survival was significantly associated with the genotype and decrease according to the number of variant alleles.
Time to metastases, PCa-specific mortality and overall survival followed similar trends (Hearn et al. 2016). While these post-prostatectomy cohorts were constructed based on eventual use on ADT, a non-selected group of 526 Caucasian patients who underwent radical prostatectomy showed no increased risk of biochemical recurrence with this same SNP (Levesque et al. 2013). Notably, in addition to its germline effects, tumors may acquire this mutation as they progress to resistance (Chang et al. 2013). A recent analysis of 216 men treated with ADT for biochemical recurrence after prostatectomy found that the HSD3B1 1245C variant was associated with shorter time to metastasis, but not time to castration resistance or survival (Hearn et al. 2017). Finally, the 1245C variant also appears to be a predictor of sensitivity to the steroidal CYP17 inhibitor abiraterone (Almassi et al. 2017).

Genomic variation in estrogen-related genes has also been reported in PCa patients (Lévesque et al. 2014). This includes somatic polymorphisms in CYP1B1, HSD17B2, SULT2B1 and COMT genes. Further, the cumulative number of alterations appears to correlate with poorer outcome (Lévesque et al. 2014). Similarly, SNPs in the CYP19A1 aromatase are associated with concomitant changes in serum estrogen levels and poorer PCa survival, but have no effect on PCa incidence (Travis et al. 2009, Kanda et al. 2015). Increased expression of CYP19A1 has been reported in PCa metastases compared to primary tumors (Cai et al. 2011).

Clinical prognostic value of serum sex steroids as biomarkers in prostate cancer patients

Multiple studies have sought to address whether serum sex steroids are related to PCa risk or Gleason grade at diagnosis, though the findings have generally been inconsistent to support an association (Severi et al. 2006, Weiss et al. 2008, Sher et al. 2009, Sun et al. 2011). Here, we discuss the role of serum steroids as biomarkers in patients who have potentially lethal PCa for which ADT is indicated.

Following initiation of ADT, higher levels of serum testosterone has been shown in multiple studies to be a biomarker for time to CRPC and PCa-specific survival (Morote et al. 2007, Perachino et al. 2010, Klotz et al. 2015, 2017, Wang et al. 2017a,b). A biological explanation for these findings is lacking, but it is notable that nadir serum testosterone differentiates the development of CRPC years later. Notably, the prognostic value of serum testosterone during ADT appears most useful immediately following treatment initiation, with no reports indicating testosterone values after one year of ADT initiation have prognostic value (Tombal et al. 2017, Wang et al. 2017a,b). One study reported that periodic surges in testosterone may impact progression (Pickles et al. 2012), but this has not been validated in other data sets. The equivalence of intermittent ADT, which results in significantly higher rises in serum testosterone levels, also raises questions about the significance of these findings. However, several small studies have found a correlation between testosterone levels on ADT and PSA values, suggesting that incomplete ADT may fuel cancer cell growth (Takizawa et al. 2010, Kawakami & Morales 2013).

In CRPC, several studies have evaluated serum testosterone during ADT as a predictive marker of response to subsequent chemotherapy, abiraterone and enzalutamide. Low testosterone prior to chemotherapy and after chemotherapy prior to abiraterone have been associated with poorer survival (Ryan et al. 2013b, Irelli et al. 2014). In addition to testosterone, lower levels of both androstenedione and DHEA-S were associated with a trend for poorer survival in men post chemotherapy, regardless of treatment arm (Ryan et al. 2013b). Other studies did not show a relationship between circulating sex steroids and response to abiraterone (Kim et al. 2014, Ryan et al. 2014, Bertaglia et al. 2017, McKay et al. 2017).

The prognostic role of serum estrogens is less clear, and their levels may be correlated with androgen levels through peripheral aromatization. A study in the pre-PSA era demonstrated significantly lower serum estrone (as well as bioavailable testosterone) in metastatic patients compared to non-metastatic PCa patients (Eriksson & Carlström 1988). Another study reported that serum E1, E2 and E1-S were commonly elevated in men with high-grade localized PCa (Giton et al. 2008). Finally, others have reported the estrogen:androgen ratio is inversely related to the risk of aggressive PCa (Black et al. 2014).

Biological effects of sex steroids within the prostate cancer tumor microenvironment

Androgens

The intratumoral de novo synthesis of androgens and intratumoral conversion of adrenal precursors to androgens represents areas of significant investigation in PCa over the last decade. Several translational studies now indicate that conversion of circulating precursors represents the most dominant path to intratumoral DHT production. Fankhauser and coworkers compared incubated fresh prostatic tissue obtained via transurethral resection of the prostate and

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analyzed the conversion of radiolabeled steroid precursors in ex vivo cultures. They found that androstenedione was the predominate source of androgens in CRPC tissue (Fankhauser et al. 2014). More recently, another group also used radiolabeled precursors and reported that adrenal androstenedione is the preferred substrate precursor for 5-alpha reduction to DHT in CRPC tissue (Dai et al. 2017). These results differed from localized prostatectomy tissue, suggesting resistant prostate tumors develop capacities to metabolize steroids, which differs from treatment-naive tumors. Finally, a recent analysis of prostate tissue from men who received neoadjuvant leuprolide acetate±abiraterone acetate identified that DHEA, epi-androstosterone and their metabolites were predominantly of adrenal origin, whereas testosterone, DHT, 3α-diols and 3β-diols were of testicular origin (Zang et al. 2017). This intratumoral androgen synthesis results in persistently higher androgen levels in the prostate: while serum androgens drop by ~95% following ADT, intraprostatic androgens decline by only 70% (Mostaghel et al. 2007).

The presence of androgens within the TME permits continued activation of the AR in PCa cells. Persistent androgen levels following ADT are associated with more aggressive PCa. In localized prostate tumors treated with 6 months of ADT prior to surgery, the relative reduction in prostatic DHT levels was greater in Gleason 6 tumors than Gleason 7–10 tumors (Nishiyama et al. 2007). Men with high grade (Gleason ≥8) had higher prostatic testosterone levels than those with Gleason ≤6 (P=0.06) (Nishiyama et al. 2007). Another study of men initiating ADT found serum DHEA-sulfate levels were correlated with Gleason score. PSA values were also correlated with all hormone values at 6 months, but not at baseline (Takizawa et al. 2010). IL-6, which is associated with advanced PCa, has also been linked with serum testosterone and DHEA among men treated with ADT (Chun et al. 2009, Komatsu et al. 2012). Steroidogenesis is higher in metastatic lesions, and this may also be stimulated by osteoclasts or extravasicle transport of CYP17 (Locke et al. 2009, Jernberg et al. 2013, Hagberg Thulin et al. 2016). Reactive, inflamed prostatic stroma may also increase the synthesis of steroidogenic enzymes, which may in turn facilitate intratumoral androgen synthesis from circulating DHEA (Piao et al. 2013).

Estrogen and progesterone

The effects of estrogens within the PCa microenvironment remain to be fully elucidated. The importance of estrogen receptors ERα and ERβ in PCa are reviewed elsewhere. Older studies implied that stromal actions of androgen and estrogens act in a paracrine manner to induce carcinogenesis via AR or ERα (Bosland 2000, Bosland & Mahmoud 2011). Studies in rat models demonstrate the combination of testosterone and estradiol, but not testosterone alone, induce prostatic cancer in 100% of rats (Noble 1977). ERβ is expressed mainly in prostate epithelial cells, but during development, may be found in both epithelium and stroma. The conversion of testosterone to estradiol within the prostate appears important, as suggested by aromatase knockout mice abrogating the induction of prostatic intraepithelial neoplasia by testosterone and estradiol treatment (Ricke et al. 2008).

In humans, comparison of microdissected PCa to benign tissue showed elevated expression of ERβ (Walton et al. 2009). ERβ appears only in human prostate epithelium, while ERα is found predominantly in the stromal compartment (Lau & To 2016). In prostatectomy specimens, higher ERα levels in tumor stroma predicted better PCa survival, while higher ERβ levels in stroma predicted sooner biochemical recurrence. Tissue aromatase levels in both epithelium and stroma were associated with longer time to biochemical recurrence or disease progression (Grindstad et al. 2016). Increased expression of aromatase has been reported in periprostatic adipose tissue, PCa cells and particularly metastatic CRPC (Hiramatsu et al. 1997, Ellem et al. 2004, Takase et al. 2006, Montgomery et al. 2008, Celhay et al. 2010). Prior clinical trials of aromatase inhibitors in PCa patients showed no benefit (Santen et al. 2001, Smith et al. 2002). Compared to Caucasian controls, African Americans have higher serum estradiol levels and high ERβ gene expression in their tumors, suggesting this may account in part for racial differences in tumor aggressivity (Rohrmann et al. 2007, Abd Elmageed et al. 2013).

The role of progesterone in the prostate TME is less clear. Its expression in microdissected PCa is lower than controls (Walton et al. 2009). In patients treated with abiraterone, accumulation of progesterone results in the selection for mutant, progesterone-activated AR clones (Chen et al. 2014). Similar to ERα, preclinical studies suggest it is the stromal PR, which is important to mediate the effects of progesterone within the TME, with the role of epithelial ER and PR in PCa progression unclear (Hobisch et al. 1997, Latil et al. 2001, Yu et al. 2013).
Sex steroids and the immune tumor microenvironment

Effect of androgens and estrogens on innate immunity

Local levels of sex steroids may alter the function of resident immune cells within the TME (Fig. 2). Indeed, differences in the immune system between sexes are largely attributable to differences in exposure to sex steroids (Trigunaite et al. 2015). The function of the immune system also changes with age, and this may be relevant to the increased incidence of prostate cancer in elderly men. The immunoreactivity of antigen-presenting cells (APCs) such as macrophages in the prostate TME is...
linked to patient outcomes (Ylitalo et al. 2016, Strömvell et al. 2017), emphasizing the importance of understanding this biology. The receptor and non-receptor-mediated effects of steroids on innate immune cells have been widely studied in experimental animal models as well as in clinical studies of many diseases (Trigunaite et al. 2015, Roved et al. 2017). Overall, androgens appear to favor the development of an immunosuppressive state, while estrogens and progesterone may have dose-varying effects on immune function. The impact of the steroidal milieu on the local immune function in prostate tumors is not clearly understood, but may contribute to its typical immunologically ‘cold’ phenotype.

Estrogens have been shown to promote the expression of HLA-DR and co-stimulating molecules in APCs (Jarrossay & Thelen 2013). Further, innate immune responses are regulated through the function of pattern recognition receptors (PRRs) on innate immune cells, and these differ by the sex (Bouman et al. 2005, Meier et al. 2009). For example, induction of TLR7 signaling causes higher production of interferon-α in women than that in men (Griesbeck et al. 2015). Spitzer and coworkers previously described the higher phagocytic activity of APCs in women with lupus compared to affected men (Spitzer 1999). Estradiol appears to exert dose-dependent effects on macrophages, with low doses favoring the production of inflammatory cytokines (IL-1β, IL-6 and TNF-α) and high concentrations favoring decreased levels (D’Agostino et al. 1999, Bouman et al. 2005). This is mediated via ERα and the nuclear factor kappa B pathway crosstalk (Härkönen & Väinänen 2006, Campbell et al. 2014). Pre-clinical studies suggest that estrogens may induce differentiation in dendritic cells and favor inflammatory cytokine expression (Bengtsson et al. 2004). However, studies in other models and in neutrophils suggest an anti-inflammatory influence (Cai et al. 2012).

Androgens in the TME may act via the AR, which is expressed in many innate immune cells, including monocytes and macrophages (Ahmadi & McCruden 2006). Testosterone has been shown to suppress inflammatory cytokine production by human macrophages (Corrales et al. 2009, Corcoran et al. 2010). In vitro, testosterone decreases the synthesis of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in macrophages and increases the secretion of CCL17 and CCL22 (D’Agostino et al. 1999). Other studies suggest that testosterone may diminish PRR toll-like receptor 4 (TLR4) expression, which is involved in polarizing macrophages to an inflammatory phenotype (Rettew et al. 2008). Cellular signaling studies indicate that testosterone regulates macrophage polarization through the inhibitory regulative Gαi-protein and Akt phosphorylation, but not via AR (Ren et al. 2017). Finally, androgens appear to decrease the function of neutrophils by diminution of secretion of the chemoattractant CXCL8 (Pioli et al. 2007).

Effect of androgens and estrogens on immunoregulatory and effector cells

While the innate immune response helps coordinate the body’s response to cancer, immunoregulatory and effector T cells are also important in the prostate TME. The T cell response is categorized in four distinct subtypes based on transcriptional factors, cytokine production and function. The IFN-γ-dominant T cell response is called Th1, while the IL-4 dominant response is called Th2; Th17 secretes the bi-functional cytokine IL-17 and regulatory T cells (Treg) secrete anti-inflammatory cytokines such as IL-10 and TGFβ.

Emerging evidence suggests estrogens may alter T cell function to favor an anti-inflammatory response, but this response is concentration dependent. A Th1 response is generally by low doses of E2, whereas high doses promote a Th2 response. Others have shown estrogens may mediate a reduction in IL-6 secretion and STAT3 activity. Interestingly, estrogen upregulates the expression of IL-10, CTLA4 and PD-1 in regulatory T cells, and mice treated with ER antagonist show a reduction of suppressive CD4+ CD25+ Treg activity (Polanczyk et al. 2004, 2007). The effect of estrogens on PCa-associated immunoregulatory cells is unclear. It appears that estradiol levels within the TME may stimulate MDSCs via ERα signaling, activating their tumor-promoting features. Ellem and coworkers described increased inflammation-related prostatic changes in detail using a murine model of aromatase gene overexpression (Ellem et al. 2004). Rat studies have also suggested estrogens are important to PCa carcinogenesis (Bosland & Mahmoud 2011). Androgens are known to suppress the activity of these immune cells (Kovats et al. 2010). Mechanisms include increasing the synthesis of anti-inflammatory cytokine mediators such as IL-10 and direct repression of nuclear factor κB (NF-κB), cJun and IRF1 or IL-12 secretion (Olsen & Kovacs 1996, Liva & Voskuhl 2001, Dunn et al. 2007, Kissick et al. 2014). It was shown in both murine and human models that androgens inhibit the polarization of Th-1 lymphocytes via the reduction of phosphorylation of the STAT4. Similarly, it was shown that Th2 lymphocytes are capable to produce steroids, decreasing the proliferation of T-helper cells and stimulating Treg production. Accordingly, androgen
deficiency in men results in a higher ratio of CD4 to CD8 T cells, with an increase of proinflammatory cytokines (IL-1β, IL-2 and TNF-α) and decreased Tregs (Musabak et al. 2003, Roden et al. 2004, Page et al. 2006). Further, a higher number of myeloid-derived suppressor cells (MDSCs) have been correlated with ADT use, through the effect of androgens on regulating MDSCs or Tregs is not clear (Vuk-Pavlović et al. 2010, Brusa et al. 2013, Davidsson et al. 2013, Di Mitri et al. 2014, Lu et al. 2017, Svoronos et al. 2017). ADT initiation has recently been demonstrated to initiate infiltration of inflammatory T cells in the Myc-CaP prostate cancer model; the induced inflammation then decreases over time. Similar to MDSCs, it remains unclear whether relative castration levels of androgens may alter the function of T cells, though some data suggest a correlation between androgen levels and regulatory T cell function.

Taken together, these studies, mostly in non-cancer models suggest that sex steroids may have an influence on the function of innate, regulatory and effector immune cells in the prostate microenvironment. An understanding of this biology may assist in the rationale development of immunotherapies for PCA patients and help identify potential biomarkers of response to immunotherapy in PCA patients.

Conclusion and future directions

The hormonal milieu within the prostate TME is tightly regulated and changes throughout PCA progression to resistance. It is also influenced by circulating and androgen precursors and circulating estrogens. While steroidogenesis is very complex, it nonetheless appears that phenotypes defined by levels of sex steroids or somatic genotype may be used to classify patients at risk of progression. However, the context is clearly important, with the utility of both biomarkers different before and after treatment.

As treatment of advanced PCA becomes increasingly complex with multiple treatments available, understanding the underlying biology becomes increasingly important to best match patients with effective treatments. For example, while androgen synthesis with abiraterone is broadly used, there remains to be clearly defined phenotypes, which best respond (or do not respond) to this treatment. Defining this phenotype in both castrate sensitive and CRPC tumors remains a priority. A better understanding of the cellular effects of sex steroids within the TME may thus facilitate rational approaches to combination therapies based on patient and tumor biology. Whether these phenotypes are best defined by tumor or host characteristics remains an open question. Given the critical roles local sex steroids play in PCA progression, accurately understanding different hormonal phenotypes may assist with personalizing treatments and utilizing rational combination therapies in patients at higher risk of progression. This includes potential combinations of AR-targeted agents with novel immunotherapies. For example, understanding the effects of androgens on APCs and immunoregulatory cells may be important to best select patients and devise combination strategies for checkpoint inhibitor therapy in PCA patients. Finally, the relationship between phenotypes defined by tumoral microenvironment factors, such as immunoreactivity and sex steroids levels and genomic classification of tumors also remains relatively unexplored. An understanding of these areas has high potential to impact patient outcomes by providing a biological framework for more effective use of existing treatments and more effective integration of novel treatments.

Declaration of interest
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