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Androgen receptor footprint on the way to prostate cancer progression

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Androgen receptor footprint on the way to prostate cancer progression

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Abstract The prostate gland is exquisitely sensitive to androgen receptor (AR) signaling. AR signaling is obligatory for prostate development and changes in AR levels, its ligands or shifts in AR mode of action are reflected in the physiology of the prostate. The AR is intimately linked to prostate cancer biology through the regulation of epithelial proliferation, suppression of apoptosis and the development of castration-resistant disease. Thus, AR is the primary therapeutic target in various prostate diseases such as BPH and cancer. Although some tumors lose AR expression, most retain the AR and have elevated levels and/or shifts in activity that are required for tumor progression and metastasis. New AR inhibitors currently in clinical trials with higher receptor affinity and specificity may improve prostate cancer patient outcome. Several events play an important role in initiation, primary tumor development and metastatic spread. Androgen receptor activity and promoter specificity change due to altered coregulator expression. Changes in epigenetic surveillance alter the AR cistrome. Both systemic and local inflammation increases with PCa progression affecting AR levels, activity, and requirement for ligand. Our current understanding of AR biology suggest that global androgen suppression may drive the development of castration-resistant disease and therefore the question remains: Does effective inhibition of AR activity mark the end of the road for PCa or only a sharp turn toward a different type of malignancy?

Keywords Androgen receptor · Prostate cancer ·
Castration resistance · Metastasis

Introduction

Prostate cancer (PCa) originates in the epithelial compartment of the prostate, an organ dependent on androgen receptor (AR) signaling for its development, maintenance and physiological function. It is not clear which early oncogenic events lead to neoplastic transformation, but due to the heterogeneity of PCa there are likely numerous initiating factors. While there is some hereditary component associated with PCa incidence, the major risk factor for the development of this malignancy is age. Two major hallmarks of aging in men are decreasing levels of testosterone and elevated levels of inflammatory cytokines, including interleukin-6 (IL-6). Systemic inflammation suppresses testosterone secretion and testosterone supplementation has been shown to reduce the levels of inflammatory cytokines in hypogonadal men [1]. On the other hand, IL-6 can activate AR at suboptimal levels of androgens and independently of its ligands potentially causing a shift in the AR cistrome and transcriptome. Analysis of 218 PCas demonstrated that the AR pathway is activated in 56% of primary and 100% of metastatic PCas [2]. Thus, it is plausible that AR is a key or at the very least an obligatory accessory protein in PCa initiation and its function continuously changes with progression. Dramatic changes occur in a variety of cell signaling processes with PCa progression (Fig. 1). In this review, we will limit our discussion to changes in AR function observed in PCa patients and mechanistically substantiated in cell lines or mouse models.

Primary tumors

In the normal prostate, AR is expressed in both epithelial and stromal compartments as well as in endothelial cells.

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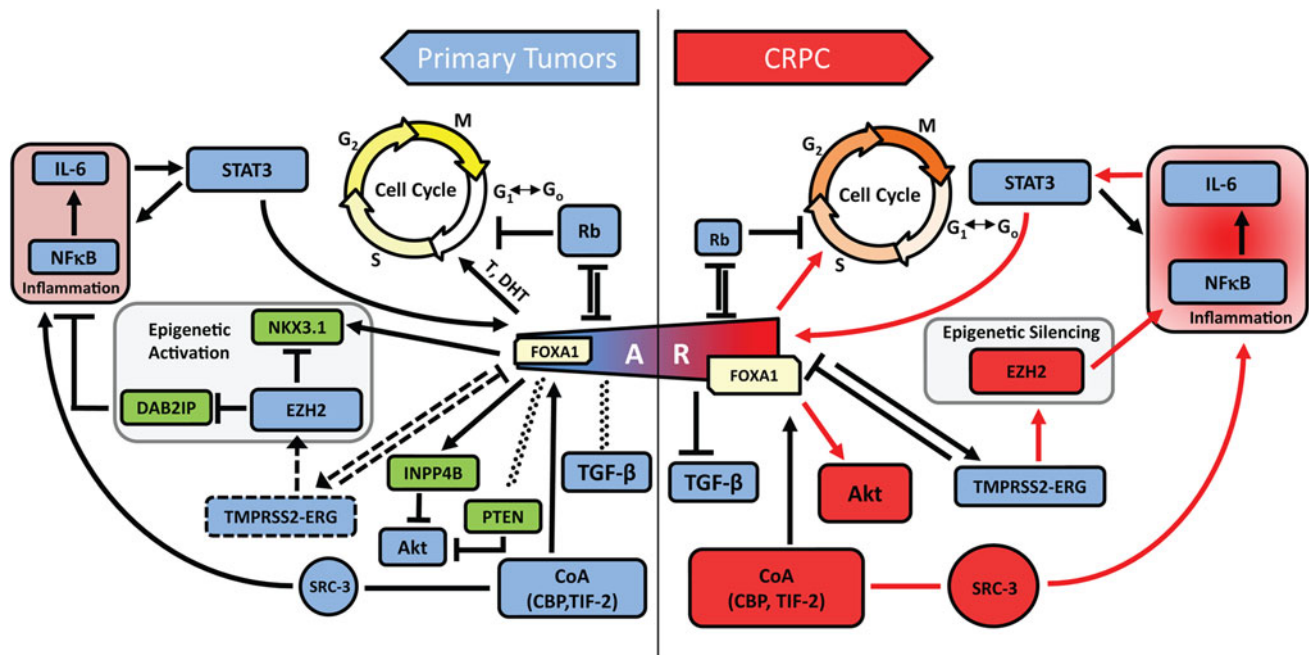


Fig. 1 Alterations in AR function in PCa and the development of CRPC. Classical ligand-dependent AR action and interplay between various intracellular signaling pathways in primary tumors (*Left*). Following androgen ablation, increased AR levels and a dramatic shift in AR function is observed ultimately leading to the development of CRPC (*Right*). Red boxes and arrows indicate increased

levels and/or activation of signaling pathways. Green boxes denote factors that are lost during the progression of PCa. The TMPRSS2-ERG fusion event is present in 60% of PCas and is indicated by the dashed lines. Dotted lines indicate cross talk with the AR

AR is a transcription factor that can be activated by a wide range of natural ligands such as testosterone, dihydrotestosterone, estradiol, as well as independently of ligand by inflammatory and growth hormone receptor pathways. Upon activation, AR binds to androgen response elements (AREs) in the promoter, enhancer and intronic regions of androgen regulated genes frequently guided by pioneer factors [3]. Once bound to regulatory regions, AR assembles a complex of coregulators that modify chromatin, general transcription machinery, and other proteins that regulate transcription [3]. During PCa progression both AR activity and target gene specificity change causing a shift in AR function from pro-differentiation to pro-proliferation.

AR levels

AR expression in primary tumors correlates significantly with an increased proliferative index and markers of aggressive disease and patients with higher levels of AR recur and ultimately die of PCa significantly faster [4, 5]. Using mouse xenograft models, Chen et al. monitored changes associated with the development of castration resistance in seven independently derived androgen dependent PCa cell lines. Remarkably, they found a single consistent change, increased expression and protein levels

of AR [6]. Overexpression of AR in PCa cells stimulated proliferation at suboptimal levels of androgens and helped overcome the inhibitory effect of bicalutamide [6]. However, overexpression of wild-type AR in luminal epithelial cells of the mouse caused high-grade prostatic intraepithelial neoplasia (PIN), without the development of overt carcinoma [7], suggesting that for PCa development both elevated AR levels and changes in activity are required.

AR Cistrome

AR binding sites on the chromatin are collectively called the AR cistrome. These sites are frequently marked by specific chromatin modifications introduced by pioneer factors such as OCT1, GATA2 and FOXA1 prior to hormone treatment. In LNCaP cells over half of the AR binding loci are also bound by FOXA1. On most of these sites, FOXA1 binds before hormone treatment and AR recruitment. Interestingly, FOXA1 ablation causes massive reprogramming of the AR cistrome: 40% of loci are preserved with increased level of AR recruitment, the remaining 60% of loci are lost, and many new AR binding sites appear, causing the AR cistrome to increase more than fourfold with corresponding changes in gene expression

[8]. A modest but highly significant reduction in FOXA1 mRNA expression in two PCa cohorts is significantly associated with metastatic PCa and poor prognosis [8]. High nuclear accumulation of FOXA1 protein is also associated with metastatic disease [9]. Thus, changes in FOXA1 expression and distribution may change the AR cistrome and consequentially its function, which is frequently observed during the progression of PCa.

AR activity

AR activity can be altered through a number of different mechanisms: receptor mutations, alternative splicing of AR mRNA, changes in the levels of coactivator and corepressors, and activation of cell signaling pathways (Fig. 1).

Mutations and splicing

A significant portion of PCas bear somatic mutations of AR that affect its ligand specificity, interaction with coregulators, dimerization, stability, and other AR properties [10]. In a mouse model, expression of an AR E231G mutant that has altered coregulator interactions caused rapid development of metastatic PCa with 100% penetrance [11]. AR splice variants (ARV), which typically lack the ligand binding domain of the receptor and do not require agonist for activity, were first identified in CWR22R PCa cells and subsequently PCa specimens. Expression of these variants is often induced by castration and associated with tumor progression to CRPC [12]. Elevated ARV levels have been observed in patients who failed hormone therapies providing survival and growth advantage by acting cooperatively with full length AR [13]. In in vitro and xenograft models, various ARVs confer both resistance to castration associated tumor atrophy and promote growth in androgen-depleted conditions [14]. Elevated ARV expression potentially disturbs cell cycle regulation and creates a shift in the AR transcriptome [14, 15]. FOXA1 directly interacts with the AR through the hinge region, which is lost or altered in many ARVs. Therefore, some mutants and splice variants may provide a survival and growth advantage in androgen-depleted conditions by shifting the AR transcriptome via circumventing FOXA1 regulation of AR genomic targeting [8].

Cell Cycle

Androgens promote proliferation through signals that modulate critical regulators of the cell cycle. For the most part, the AR regulates the cell cycle through induction of signals that regulate G1-S phase transition through the promotion of G1 cyclin-dependant kinases (CDKs) and inhibition of the retinoblastoma (Rb) tumor suppressor

gene [16]. Androgens also regulate the transcription and turnover of the cell cycle regulators p21^{Cip1} and p27^{Kip1}. The M-phase checkpoint protein UBE2C, a direct target of the AR, is significantly overexpressed in CRPC. In addition, AR displays ligand independent recruitment to the UBE2C loci in ablation-resistant PCa cell lines concurrent with elevated UBE2C expression [17].

Coactivators

Coregulators are integral to the normal function of steroid receptors and the development and progression of a wide spectrum of human diseases [18]. AR coregulators stimulate or decrease AR activity in a promoter specific manner [19]. Therefore, changes in particular coregulator expression and regulation will have distinct effects on AR target genes and the AR transcriptome.

AR is potentiated by the p160 family of coactivators that include SRC-1 (NCOA1), TIF2 (NCOA2), and SRC-3 (AIB1, NCOA3). In the normal prostate, SRC-1 expression is uniform throughout the prostate but varies considerably between men. Levels of SRC-1 do not change with the development of PCa but men that have higher SRC-1 expression will develop more aggressive PCas [20]. TIF2 expression in normal prostate is low to undetectable. With primary PCa, development expression of TIF2 increases from low to high-grade tumors and recurrent CRPCs have the highest uniformed expression of TIF2 [21]. The AR directly binds to the TIF2 promoter and an intron and represses TIF2 expression. Reduced androgen levels increase TIF2 expression and it becomes available to potentiate AR and other transcription factors stimulating both AR dependent and AR independent proliferation [21]. Thus, androgen ablation itself may cause increased TIF2 expression and contribute to the development of CRPC. The TIF2 gene is positioned at 8q13.3 in close proximity to MYC at 8q24.21; these regions are most frequently amplified in PCa [2]. Consistent with our protein expression studies, Taylor et al. found positive copy number alterations through amplification and overexpression of TIF2 in 8% of primary tumors and 37% of metastases [2]. Significantly, primary tumors with amplification of TIF2 display an increase in AR signaling [2]. SRC-3 expression is similar throughout the same prostate but varies greatly between patients [22]. Similar to SRC-1, elevated levels of SRC-3 in normal prostate is a predictor of the development of more aggressive PCa but its expression is significantly increased with the development of primary tumors [22]. In addition to activating AR transcriptional activity, SRC-3 coactivates AP1 and PEA3 transcription factors [23]. Akt is a direct transcriptional target of AP1 and increased SRC-3 expression increases total and phospho-Akt levels;

increased expression of SRC-3 in PCa correlates significantly with pAkt levels in PCa specimens as well as increased proliferation and reduced apoptosis [22, 23]. In addition, SRC-3 stimulates cellular motility by activating focal adhesion kinase signaling and invasion by activating AR, AP1 and PEA3 dependent expression of matrix metalloproteinases 2 and 13 [23].

Inflammation

As men age, circulating levels of inflammatory cytokines, including IL-6, tumor necrosis factor α (TNF- α) and interleukin-1 β (IL-1 β), increase along with PCa incidence [1, 24]. Particular focus has been on IL-6, since IL-6 has been shown to potentiate AR function. Regions of focal atrophic prostatic epithelium in aging men are common and are often associated with inflammation. These lesions, termed proliferative inflammatory atrophy (PIA), typically display increased epithelial proliferation, are associated with PIN and proposed to be precursors of PCa [24]. In benign prostate epithelium, IL-6 is expressed in basal cells, but in PIN and PCa its expression is detected in the secretory epithelium [25]. The highest circulating levels of IL-6 are observed in patients with CRPC and metastatic disease [26]. Increased IL-6 levels are associated with markers of PCa progression and poor prognosis such as androgen independent growth, resistance to chemotherapeutic drugs and neuroendocrine differentiation. In vitro studies show that IL-6 can both inhibit and promote the proliferation of AR expressing LNCaP PCa cells depending on the cellular context and ligand availability [26, 27]. IL-6 activates ligand independent AR activity via STAT3 and overexpression of IL-6 can protect LNCaP cells from growth arrest and apoptosis induced by androgen starvation [27].

One of the major conduits of inflammation is the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) family of transcription factors. Elevated NF- κ B activity in primary PCas is associated with poor prognosis and reduced time to biochemical recurrence [28]. Continuous activation of the NF- κ B pathway in vivo inhibits prostate regression following castration, maintains nuclear AR and sustains epithelial proliferation [28]. In human PCa cell lines, NF- κ B signaling interferes with cell cycle arrest and apoptosis mediated by androgen deprivation and stimulates AR ligand independent transcriptional activity and recruitment of coactivators such as p300 [29]. Interestingly, processing the NF- κ B family member p100 to produce p52 requires STAT3, a downstream signaling target of IL-6 and conversely IL-6 is a NF- κ B target. Therefore, prostatitis and PCa associated inflammation likely aid in the development and progression of PCa by modulating AR function.

AR, reactive stroma, and PCa progression

In the normal stroma, AR stimulates secretion of paracrine factors that regulate the proliferation and survival of the epithelium. The stromal compartment of the prostate undergoes significant changes with PCa progression and both expansion and loss of the stroma signals poorer prognosis [30]. Recent studies indicate that reduced peritumoral expression of stromal AR is associated with negative clinical parameters and significantly earlier relapse following radical prostatectomy [31]. Loss of stromal AR likely promotes cancer progression through deregulation of stromal factors required for normal growth and differentiation of the epithelium. In PCa, stroma often becomes reactive characterized by a reduction in well differentiated smooth muscle cells and increased deposition of extracellular matrix and collagen fibrils. Patients who have more than 85% of intra-tumoral reactive stroma are at 17.13 times higher risk of biochemical recurrence [32]. Reactive stroma promotes invasion and metastasis of PCa. A number of stromal paracrine factors have been implicated in PCa, including transforming growth factor β (TGF- β), which is known to cross-talk with the AR. TGF- β and AR signaling cooperate to maintain the differentiated state of the stroma in the benign prostate. In the normal prostate, stromal TGF- β signaling is anti-proliferative, pro-apoptotic and enhances differentiation of the luminal epithelium. In malignant epithelial cells, AR suppresses TGF- β receptor II (T β R-II) transcription and reduces TGF- β 1 driven apoptosis [33]. Furthermore, AR impedes TGF- β 1 signaling in the epithelium through direct interaction and transcriptional suppression of SMAD3 [33]. These data suggest that AR action in malignant epithelial cells provides a growth advantage by suppressing TGF mediated pathways. Therefore, androgen ablation therapies, which suppress epithelial AR mediate proliferation, also likely suppress beneficial stromal AR activity and contribute to the progression and establishment of CRPC.

Metastatic dissemination

It is becoming evident that tumor dissemination occurs early in PCa development. The first wave of cancer cell shedding occurs during primary tumor growth. The abundance of circulating tumor cells (CTC) is predictive of patient prognosis [34]. CTC analysis revealed that these cells have the same TMPSS2-ERG fusion status as primary tumor but increased heterogeneity of AR levels and PTEN loss [35], suggesting that these alterations may be subsequent to the fusion event. If indeed TMPRSS2-ERG fusion occurs early in tumorigenesis, its expression is then significantly increased by AR signaling. Overexpression of

the oncogenic transcription factor ERG under an AR responsive promoter causes profound changes: Reduction in AR expression, attenuation of AR activity at gene specific loci, activation of NF- κ B, and induced expression of epigenetic factors such as the methyltransferase EZH2 that in turn epigenetically silences differentiating factors and tumor suppressors, such as *Nkx3.1* and *DAB2IP* [36, 37]. While a number of different fusions events between TMPRSS2 and ETS family members have been identified and implicated in the progression of PCa, TMPRSS2-ERG is the most common and has the biggest impact on prognosis.

The luminal secretory epithelium is characterized, in part, by polarized orientation, cohesive extracellular interaction between like cells, and relative immobility of those cells in respect to the basal membrane. This phenotype is maintained by adhesion molecules such as α - and β -catenin, which connect the actin cytoskeleton to intracellular domains of cadherins. Loss or down regulated of critical adhesion molecules enables cells to detach from their epithelial scaffold and move more freely within the extracellular matrix. When β -catenin translocates to the nucleus it can coactivate both AR and TCF/LEF transcription factors that induce epithelial to mesenchyme transition (EMT) markers Slug and Twist. Following castration in mouse, PCa xenografts β -catenin relocated to the nuclei of LNCaP cells. Survival rates are significantly shorter among PCa patients if β -catenin is not exclusively localized to the cell membrane and β -catenin expression is lost in metastasis [38, 39]. Multiple studies have shown that loss of E-cadherin expression and translocation of β -catenin from the leading edge of the cell to the nucleus accompanies the transition to a mesenchymal phenotype in vivo [40–42]. Although some controversy still exists concerning the incidence of EMT in cancer, the concept is supported by the observation of aberrant E-cadherin and β -catenin expression in metastatic PCas [40, 43].

Role of AR in development of castration-resistant prostate cancer (CRPC)

Following the spread of PCas the standard of care is androgen ablation. Ablation is effective at first, but PCa almost always recurs as CRPC, suggesting that inhibiting AR activity activates pro-survival mechanisms. While it is not known which mechanisms are activated in individual PCa patients, expression data implicates several key pathways. As mentioned previously, castration elevates levels of TIF2 and p300, and ligand independent activators such as IL-6. In addition, one of the negative regulators of Akt action, INPP4B, is a direct AR target gene [44] and is likely lost during castration. Indeed, activation of Akt

signaling follows androgen ablation in men. In mouse models, activation of Akt can cause development of PIN even in the absence of AR signaling [45] while synergistic activation of Akt and AR is sufficient to cause frank carcinoma [46]. In addition, TMPRSS2-ERG fusion causes increased EZH2 signaling, associated with metastatic spread and recurrence.

Conclusions

The AR clearly represents a central hub in the regulation of normal prostate biology and the development, progression, therapeutic resistance and metastatic spread of PCa. Targeting the AR remains the central focus of PCa therapeutics and basic research. Despite this central role, therapeutic targeting of the AR has yet to prove fully successful. Our current and ever evolving understanding of the AR its cross-talk with intracellular signaling pathways and its compartmental functions will hopefully shed light on selective treatment targets. The selective targeting of key regulators and/or mediators of AR function in addition to or as an alternative to global AR ablation should provide customized patient care and improved outcomes.

Acknowledgments We apologize to our colleagues for our inability to cite and discuss numerous important studies due to length restrictions.

Conflict of interest The authors declare that they have no conflict of interest.

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