Androgen receptor co-regulatory networks in castration-resistant prostate cancer

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Abstract

Androgen and the androgen receptor (AR) are critical effectors of prostate cancer. Consequently, androgen deprivation therapy is typically employed as a first-line treatment for prostate cancer patients. While initial responses are generally positive, prostate tumors frequently recur and progress to a lethal form known as castration-resistant prostate cancer (CRPC). Recently, considerable effort has been directed toward elucidating the molecular mechanisms of CRPC. Results from both preclinical and clinical studies suggest that AR-mediated signaling persists and remains functionally important in CRPC despite the elimination of androgens. Understanding the role of this pathway in the development of resistance will therefore be critical to identify alternative diagnostic markers as well as more effective therapies for the treatment of CRPC. Using next-generation sequencing and other high-throughput approaches, numerous groups are beginning to identify the key differences in the transcriptional regulatory and gene expression programs between androgen-dependent and CRPC. A number of mechanisms have been proposed for the differences and these mostly involve alterations to components of the AR co-regulatory network. In this review, we summarize current knowledge on co-regulators of the AR and discuss their potential roles in CRPC. It is anticipated that a deeper understanding of these factors will undercover new targets that can assist in the diagnosis and treatment of CRPC.

Key Words
- androgen receptor
- co-regulators
- prostate cancer
- castration resistant

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Introduction

Prostate cancer is the second leading cause of cancer death among men in the USA (Siegel *et al*. 2012). The androgen receptor (AR), a member of the nuclear receptor (NR) superfamily of ligand-activated transcription factors, is a key regulator of normal prostate function as well as cancer development (Buchanan *et al*. 2001, Lonergan & Tindall 2011). Multiple studies have now revealed that AR-regulated genes participate in various cellular processes that contribute to the initiation and progression of prostate cancer (Bolton *et al*. 2007, Jariwala *et al*. 2007, Massie *et al*. 2011). Based on these findings, blocking AR signaling through androgen deprivation or by specific inhibitors such as bicalutamide (Casodex AstraZeneca, London, UK) has been utilized as standard treatments for patients with advanced prostate cancers. Despite initial good responses, tumors invariably recur and develop into a lethal form of the disease, known as castration-resistant prostate cancer (CRPC).

Extensive investigation into the molecular mechanisms of castrate resistance in recent years indicates that AR signaling persists in CRPC and continues to play a pivotal role in the progression of the disease (Attard *et al*. 2008,

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Snoek *et al*. 2009, Andersen *et al*. 2010). For instance, microarray analysis of prostate cancer tumors at different stages of progression revealed that the overall pattern of gene expression for CRPC tumors is more similar to untreated (hormone-naive), androgen-dependent primary cancers rather than tumors that have been subjected to androgen ablation treatment, a finding that suggests there has been a reversal of gene expression induced by androgen deprivation and subsequent reactivation of AR signaling under androgen-deplete conditions in CRPC (Holzbeierlein *et al*. 2004). Moreover, AR signaling has been shown to become constitutively active in situations where the AR gene is altered such as through amplification, rearrangements, or splicing (Chen *et al*. 2004, Dehm *et al*. 2008, Li *et al*. 2011, Zhang *et al*. 2011*a*), or in other instances, AR signaling can be sustained even in the absence of a ligand through cross talk with other signaling pathways, including IL6 and PI3K/Akt (Wen *et al*. 2000, Ueda *et al*. 2002). While it is accepted that continued activation of AR signaling axis facilitates the development of CRPC, a critical question that remains is whether diverse AR-mediated transcriptional program and gene networks are in play in androgen-dependent and CRPC. While the early study by Holzbeierlein *et al*. (2004) reported similarities in overall gene profiles of androgen-dependent and CRPC, recent studies are beginning to identify important differences in the expression of specific genes following transition of prostate cancer from an androgen-dependent state to one that is androgen-independent, reflecting distinct AR transcriptional regulatory programs in the two stages of prostate cancer (Tomlins *et al*. 2007, Wang *et al*. 2009, Decker *et al*. 2012, Sharma *et al*. 2012).

The binding of AR to chromatin and the transcriptional activity of the receptor are regulated by numerous factors in a series of coordinated events (Cheung & Kraus 2010, Chng & Cheung 2012). Deregulation of either the expression or the activity of these factors is therefore likely to result in alterations to the AR cistrome and transcriptome, which may contribute to or even govern the progression of prostate cancer. Addressing how these factors regulate AR signaling under both hormone-responsive and castrate-resistant setting will be a paramount to the development of more specific biomarkers as well as much needed improved therapies for the detection and treatment of CRPC. Herein, we will review current findings on how AR co-regulatory networks function to modulate the transcriptional output of AR and how changes in the AR transcriptome may facilitate the development of CRPC.

AR co-regulatory networks

Similar to other members of the NR family, AR consists of structurally and functionally distinct domains, including a DNA binding domain, a ligand-binding domain, two transcriptional activation domains, and a hinge region that harbors a nuclear localization signal (Gelmann 2002, Centenera *et al*. 2008, Cheung & Kraus 2010). Upon androgen stimulation, AR translocates from the cytoplasm into the nucleus, dimerizes, and binds to specific DNA recognition sequences termed androgen response elements (AREs), where it subsequently recruits a series of co-regulatory proteins that either enhance (coactivators such as p160/SRC and CBP/p300) or repress (corepressors such as NCoR and SMRT) the transcription of target genes (Bennett *et al*. 2010). Co-regulator proteins can influence AR-mediated transcription in a number of ways, including coordinating changes in the structure of the chromatin or facilitating interactions with the RNA polymerase transcriptional machinery (Rouleau *et al*. 2002, Shang *et al*. 2002, Louie *et al*. 2003).

The recent development of high-throughput technologies has fundamentally altered how prostate cancer research is performed by creating new approaches that allow the interrogation of AR-mediated transcription on a genome-wide level (Chng & Cheung 2012). For example, by coupling chromatin immunoprecipitation with microarray (ChIP-Chip) as well as next-generation sequencing (ChIP-Seq), researchers have been able to obtain high-resolution genome-wide binding site maps of AR and other transcription factors in prostate cancer cells (Wang *et al*. 2007, Tan *et al*. 2012). One notable finding from the mapping of AR binding sites (ARBS) is that AR is preferentially distributed at distal regulatory regions of genes (Tan *et al*. 2012), a result consistent with previous reports showing that AR may regulate transcription through chromatin looping (Wang *et al*. 2007, Makkonen *et al*. 2009). Genome-wide analysis of ARBS also revealed that only a small portion of binding sites contains the canonical class I NR motif (5′-AGAACANNNTGTTCT-3′, allowing up to two positions to vary from the palindromic consensus with three nt spacing) (Verrijdt *et al*. 2003), whereas most sites contain only a half-site of the ARE (Wang *et al*. 2007). This is in contrast to other NRs such as estrogen receptor α and peroxisome proliferator-activated receptor γ, whose binding sites predominantly contain their cognate consensus response elements (Lin *et al*. 2007, Nielsen *et al*. 2008, Welboren *et al*. 2009, Tan *et al*. 2011).

The low occurrence of canonical AREs at ARBS suggests that the binding of AR to chromatin is likely

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dependent on additional transcription factors. Indeed, bioinformatic analysis of ARBS revealed that there is an enrichment of DNA sequences for motifs of numerous transcription factors (Massie *et al*. 2007, Wang *et al*. 2007, Zhang *et al*. 2011*b*, Tan *et al*. 2012). For example, motifs for pioneer factors including FoxA1, GATA2, and OCT-1, as well as other collaborative factors such as ETS-1, AP4, and NKX3-1, are preferentially distributed near the center of ARBS (Massie *et al*. 2007, Wang *et al*. 2007, Zhang *et al*. 2011*b*, Tan *et al*. 2012). While most of these collaborative factors function to augment AR transcription, a distinctive feature of pioneer factors is their ability to associate with condensed chromatin independently of other factors and to directly modulate chromatin accessibility (Zaret & Carroll 2011). Importantly, the interaction of pioneer factors with chromatin occurs prior to androgen treatment (Wang *et al*. 2007, Tan *et al*. 2012), implying that these factors can potentially direct the recruitment of AR to chromatin even at low or no androgen levels. Collectively, the combined actions of coactivators/corepressors, pioneer factors, and collaborative factors form an important part of a transcriptional co-regulatory network that functions to coordinate AR-mediated gene expression.

Distinct genomic and transcriptional programs in CRPC

Until recently, our understanding of how AR regulates transcription in prostate cancer has been limited mostly to studies using prostate cancer cell lines. While we have gained much insight into the mechanism of AR-dependent transcription from these studies, there are clearly limitations of what information we can obtain with cell lines. With the latest innovations in NGS Technologies, we now have the ability to generate and analyze genomic information from tumors of prostate cancer patients. The findings from such studies have greatly advanced our knowledge on the dynamics of AR transcription and shed new light on the changes in genomic and transcriptional landscapes of AR in prostate cancer, in particular CRPC.

For example, in order to define the genomic landscape of AR in human prostate tumors, especially those that have become castrate resistant, Sharma *et al*. (2012) performed ChIP-Seq analysis of AR on prostate tissues obtained from ten patients with prostate cancer (five castrate-resistant, two androgen deprivation treatment-responsive, and three untreated) and two men with benign prostate hyperplasia. Importantly, their study mapped thousands of ARBS in CRPC tissues, many of which had not been previously identified in prostate cancer cell lines. Moreover, they found that while ARBS in both untreated and CRPC were predominantly located in distal intergenic regions, there was a significantly larger portion of ARBS at promoters in CRPC compared with untreated prostate cancer, which suggests that there is a change in AR binding profiles in these two forms of the disease (Sharma *et al*. 2012). Finally, using Genomic Regions Enrichment of Annotations Tool (GREAT) analysis, the authors showed that ARBS in untreated prostate cancer were enriched for aerobic respiratory genes, whereas ARBS in CRPC were mostly associated with genes involved in metabolic pathways such as glucose and triglyceride homeostasis (Sharma *et al*. 2012). The differential activation of biological pathways further supports a divergent transcriptional program regulated by AR in CRPC, which likely serves to meet the changing needs of cancer cells as the disease progresses. Consistent with the patient tumor findings, others have also reported distinct AR binding events in CRPC cell line models, C4-2B and LNCaP-abl (Decker *et al*. 2012). Specifically, AR binding events in CRPC activated mitotic cell cycle genes that help to drive cell proliferation (Decker *et al*. 2012).

What could be the underlying mechanism behind the reprogramming of AR action in CRPC? One possibility could be changes in the recruitment of collaborative factors to ARBS. For instance, ARBS in untreated prostate cancer were significantly enriched for motifs of known AR collaborative factors such as FoxA1 and NF-1, whereas ARBS in CRPC tissues were instead enriched for E2F, MYC, and STAT motifs, in addition to forkhead and NF-1 motifs (Sharma *et al*. 2012). Notably, Sharma *et al*. (2012) elegantly demonstrated that treatment of androgen-dependent LNCaP cells with a cocktail of cytokines, which have been previously shown to stimulate signaling by E2F, MYC, and STAT, redirected AR binding to sites originally occupied only in CRPC tissues. Given that the cytokine IL6 is significantly elevated in the serum of CRPC patients (Drachenberg *et al*. 1999) and promotes androgen-independent growth of prostate cancer cells (Lee *et al*. 2003), the findings from Sharma *et al*. suggest that E2F, MYC, and STAT could be potential collaborative factors that determine the cistrome of AR in CRPC. Thus, it will be important in future work to address the molecular interactions between these factors and AR in CRPC tissues or cell lines.

Collectively, the above findings suggest that the acquisition of new ARBS and AR-regulated genes during disease progression is likely orchestrated by modulations to the repertoire and activity of AR collaborative factors;

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however, the specific function of these collaborative factors in CRPC remains to be evaluated. In the following sections, we will highlight the effects of selected collaborative factors on AR as well as discuss their roles in CRPC.

Reprogramming of the AR cistrome by FoxA1

FoxA1, a member of the forkhead family of transcription factors, is a pioneer factor that has been shown to facilitate the recruitment of NRs to their cognate DNA binding sites (Gao *et al*. 2003, Carroll *et al*. 2005, Lupien *et al*. 2008, Hurtado *et al*. 2011). Based on this property, the absence or depletion of FoxA1 should lead to a loss of AR binding to chromatin. However, recent work from two independent groups showed that siRNA knockdown of *FoxA1* in prostate cancer cells surprisingly led to a global redistribution of ARBS that included an extensive gain in novel AR binding loci (Sahu *et al*. 2011, Wang *et al*. 2011). Thus, it appears that FoxA1 can regulate the DNA binding of AR in prostate cancer cells via two distinct opposing mechanisms, by either facilitating or transrepressing the binding of AR to chromatin (Sahu *et al*. 2011, Wang *et al*. 2011). Notably, Wang *et al*. (2011) observed an increase in the production of enhancer-templated non-coding RNAs at ARBS as a result of FoxA1 depletion and this was accompanied by corresponding changes in the expression of nearby genes, which suggests that the new ARBS are transcriptionally functional.

Despite reporting similar findings on the ability of FoxA1 to regulate the genomic landscape of AR, the two groups differed on the impact of FoxA1 expression on the prognosis of patients with prostate cancer. Specifically, Wang *et al*. (2011) showed that FoxA1 expression was low in poor prognostic CRPC, while Sahu *et al*. (2011) found a positive association between low FoxA1 expression and survival after radical prostatectomy. We postulate that the discrepancy between the two studies on the effect of FoxA1 expression on the prognostic outcome of prostate cancer cases may be attributed to the differential functions of FoxA1 in androgen-dependent and androgen-independent prostate cancer. For instance, previous studies showed that in the presence of castrate serum androgen levels, AR transcriptional activity remains intact in CRPC and AR signaling can be rapidly reactivated through intratumoral androgen synthesis (Montgomery *et al*. 2008, Attard *et al*. 2009, Cai *et al*. 2011*a*). Under this circumstance, a low level of FoxA1 expression may bring about a release of its transrepression activity on 'gained' ARBS, resulting in a transcriptional program that may contribute to cancer progression and thus associated with poor prognosis. On the other hand, in the absence of androgen, FoxA1 has been shown to be essential for the recruitment of AR to selective enhancers, such as those of M-phase genes (Wang *et al*. 2009). Most notably, FoxA1 function was required for *UBE2C* expression in LNCaP-abl cells, which accelerated M-phase transition (Wang *et al*. 2009). As such, in an androgen-deplete environment, a low level of FoxA1 may in fact help to suppress tumor growth and thus correlate with good prognosis in prostate cancer patients. Furthermore, it must be understood that the pathogenesis of CRPC is not dependent on FoxA1 alone, but likely involves a synergistic integration of multiple signaling pathways. For example, studies have implicated the FoxA1 and insulin-like growth factor axis in the progression of prostate cancer (Imamura *et al*. 2012, Potter *et al*. 2012). Hence, the contrasting prognostic effects of FoxA1 protein expression in different cohorts are therefore not unexpected, given the inter-patient heterogeneity of CRPC. Taken together, FoxA1 is a context-dependent dual-function pioneer factor that may be critical to the development of castrate resistance.

Modulation of androgen dependence by ERG

Chromosomal rearrangements between the regulatory region of the androgen-responsive gene *TMPRSS2* and members of the *ETS* family of transcription factors such as *ERG* or *ETV1* are commonly found in prostate cancers (Tomlins *et al*. 2005). The most prevalent ETS gene fusion *TMPRSS2:ERG* occurs in ~50% of prostate cancers and its introduction into primary or immortalized benign prostate epithelial cells results in markedly increased invasiveness properties of the cells, suggesting that ERG may help to drive the progression of prostate cancer (Tomlins *et al*. 2008, Magi-Galluzzi *et al*. 2011). Moreover, while the level of *TMPRSS2:ERG* expression decreases following androgen deprivation therapy, it subsequently returns to pre-castration levels in recurrent cancers, consistent with the reactivation of AR signaling in CRPC (Cai *et al*. 2009).

To examine the role of ERG in the pathogenesis of CRPC, Rickman *et al*. (2010) searched for genes whose expressions were significantly altered in CRPC tumors harboring the *ERG* rearrangement in comparison to hormone-naive prostate cancers. From their analysis, the authors identified *TFF3*, which encodes for the human intestinal trefoil factor 3 protein as the most differentially regulated gene between the two groups (Rickman *et al*. 2010). In mechanistic studies, they showed that *TFF3* was directly regulated by ERG in both hormone-naive and

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CRPC, but more importantly, ERG inhibited the expression of *TFF3* in the presence of androgen while stimulating it in an androgen-free setting (Rickman *et al*. 2010). This finding suggests that ERG can exert opposing effects on the regulation of *TFF3* depending on the level of androgen signaling in the microenvironment of the cancer cell (Rickman *et al*. 2010). While the specific role of TFF3 in CRPC is still unclear, induction of *TFF3* expression by ERG enhanced the invasive potential of CRPC cells (Rickman *et al*. 2010). Collectively, ERG appears to promote castrate resistance in part by switching on an AR suppressed pathway, leading to aggressive tumor growth even in conditions where cells are deprived of androgen.

To determine the extent of AR and ERG collaboration in prostate cancer, two groups recently performed ChIP-Seq analyses of AR and ERG on prostate cancer cell lines and tumor samples (Yu *et al*. 2010, Chng *et al*. 2012). Overall, global analyses of these two factors revealed that there is a substantial overlap in binding between these two transcription factors suggesting a genome-wide transcriptional collaboration between AR and ERG in regulating androgen-stimulated gene transcription that extends beyond *TFF3*. Notably, Yu *et al*. (2010) observed that ERG can disrupt AR signaling by directly inhibiting the transcription of the AR gene as well as suppressing the expression of AR target genes at specific loci. In support of this proposed attenuation of AR signaling by ERG, our group showed that depleting ERG in prostate cancer cells resulted in enhanced AR binding and transcriptional activity (Chng *et al*. 2012). Moreover, we observed that ERG directly repressed the AR-mediated upregulation of the cytoskeletal gene, Vinculin, whose depletion led to an increase in the invasive capability of prostate cancer cells (Chng *et al*. 2012). In addition to its repressive role, ERG may also act as an enhancer of AR function depending on the cellular context (Chen *et al*. 2013). Taken together, these findings implicate ERG in prostate cancer progression by modulating AR transcriptional activity and regulating genes that may inhibit tumorigenesis.

Interestingly, ERG can also function independently of AR signaling in prostate cancer cells (Yu *et al*. 2010). Ectopic overexpression of ERG in prostate cancer cells dramatically increased cell invasion and proliferation even in the absence of androgen stimulation or when AR expression was depleted by siRNA (Yu *et al*. 2010). The ability of ERG to promote prostate tumorigenesis independent of AR was further substantiated by a study which demonstrated that ERG can act synergistically with an aberrant PI3K pathway to induce invasive prostate adenocarcinoma in a mouse model (Zong *et al*. 2009). Collectively, Yu *et al*. (2010) proposed a working model for the role of ERG in CRPC, whereby ERG promotes castrate resistance by simultaneously suppressing AR pro-differentiation programs and regulating AR-independent oncogenesis.

Dual modulation of androgen-dependent transcription by enhancer of zeste homologue 2

Enhancer of zeste homologue 2 (EZH2), the catalytic subunit of the polycomb repressor complex 2 (PRC2) which mediates the trimethylation of H3K27, has been implicated in the progression of a variety of cancers, including bladder, breast, and prostate (Chang & Hung 2012). In prostate cancer, the mRNA and protein levels of EZH2 are frequently found over-expressed and an elevated level of EZH2 has been linked to more aggressive tumors and poorer patient prognosis (Varambally *et al*. 2002). Moreover, EZH2 has been proposed to regulate the progression of the disease by promoting the proliferation of prostate cancer cells, likely through H3K27me3 and the subsequent repression of target genes (Varambally *et al*. 2002).

To examine the functional relevance of EZH2-mediated H3K27me3 in prostate cancer progression, Yu *et al*. (2007, 2010) took a genome-wide approach and generated a series of global PRC2 and H3K27me3 binding profiles in prostate cancer cell lines as well as tissues. In their initial study, the authors used the ChIP-Chip assay to map genomic regions occupied by SUZ12, a subunit of PRC2, and H3K27me3 (Yu *et al*. 2007). Notably, they observed that a significant portion of H3K27me3-marked genes was occupied by PRC2 in both LNCaP cells and human tumors (Yu *et al*. 2007). Moreover, a large number of these genes were downregulated and associated with poor clinical outcome (Yu *et al*. 2007). Interestingly, Oncomine Molecular Concepts Map (MCM) analysis showed that H3K27me3-occupied genes in advanced prostate cancer tissue were enriched for H3K27me3-occupied genes in embryonic stem cells, including developmental regulators and signaling molecules, thus implicating PRC2 in the maintenance of prostate cancer cells in an undifferentiated state, much like that of stem cells (Yu *et al*. 2007). In their subsequent work, Yu *et al*. (2010) applied the ChIP-Seq Technology and MCM analysis to investigate the possible interactions among AR, ERG, and EZH2 in prostate cancer. From their work, they found that ERG binds to and regulates the expression of genes previously reported to be targets of EZH2 (Yu *et al*. 2010). In addition, they showed that ERG also directly activates the transcription of *EZH2*, which consequently leads to the downregulation of EZH2-repressed genes

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(Yu *et al.* 2010). Collectively, the epigenomic work by Yu and colleagues in prostate cancer cells indicates that EZH2, in coordination with ERG, may contribute to the progression of prostate cancer via epigenetic silencing of tumor-suppressing genes and the induction of a stem cell-like dedifferentiation program (Yu *et al.* 2007, 2010).

While the repressive transcriptional activity of EZH2 is well documented in prostate cancer (Chen *et al.* 2005, Cao *et al.* 2008, Ren *et al.* 2012, Zhao *et al.* 2012), a recent report by Xu *et al.* (2012) suggests that EZH2 may also be capable of functioning as a positive regulator of transcription, specifically in the context of CRPC. In their work, the authors examined the effects of depleting EZH2 on global gene expression in androgen-dependent and independent prostate cancer cells (Xu *et al.* 2012). Knockdown of *EZH2* in the androgen-dependent LNCaP cell line resulted in a similar number of genes that were either up- or downregulated, but in the androgen-independent cell line, LNCaP-abl, knockdown of *EZH2*, produced a larger portion of genes that were markedly downregulated (Xu *et al.* 2012). Interestingly, the authors observed that the expression levels of EZH2-stimulated genes in CRPC were more positively correlated with *EZH2* expression than EZH2-repressed genes (Xu *et al.* 2012). These results led the authors to hypothesize that EZH2 may have the ability to switch from being a transcriptional repressor to a transcriptional activator when the prostate cancer transitions to CRPC. In support of their hypothesis, Xu *et al.* (2012) showed that EZH2 binding sites in LNCaP-abl cells lacked H3K27me3 modifications but instead were enriched for the active histone marks H3K4me2 and H3K4me3 as well as RNA polymerase II. Moreover, upon EZH2 depletion, the levels of active marks at these sites decreased, further supporting the role of EZH2 as a transcriptional activator (Xu *et al.* 2012).

How can EZH2, a protein known to be a transcriptional repressor, also function as a transcriptional activator? Previous studies showed that a fully functional repressive PRC2 complex consists of EZH2 and at least two other subunits, SUZ12 and EED (Cao & Zhang 2004, Pasini *et al.* 2004, Montgomery *et al.* 2005). However, findings by the Brown Laboratory showed that the transcriptional activating effect of EZH2 is independent of the SUZ12 and EED subunits but requires the intact methyltransferase activity of EZH2 (Xu *et al.* 2012). Moreover, motif analysis and knockdown experiments by the group suggest that EZH2 and AR cooperatively recruit each other to specific loci and EZH2 likely exerts its activation function by modulating the methylation status of AR or AR-associated proteins (Xu *et al.* 2012). Finally, Brown and colleagues demonstrated that the functional switch that turns EZH2 from a transcriptional repressor to a coactivator is mediated by its phosphorylation at serine-21, a modification that appears to be regulated by the PI3K/Akt signaling pathway (Xu *et al.* 2012). Of note, the site-specific phosphorylation of EZH2 was shown to be necessary for the induction of androgen-independent growth in LNCaP cells and the support of continued androgen-independent growth in LNCaP-abl cells (Xu *et al.* 2012). Taken together, EZH2 functions in a noncanonical manner in CRPC to activate AR target gene expression that may promote oncogenesis in the castrate-resistant setting.

Rewiring of AR signaling axis by cyclin D1b

Cyclin D1a, one of the two splice isoforms of cyclin D1, binds directly to AR and inhibits its transcriptional activity (Knudsen *et al.* 1999, Reutens *et al.* 2001, Comstock *et al.* 2011). Despite being frequently over-expressed in many tumor types, cyclin D1a is rarely deregulated in prostate cancer and has not been reported to have any independent prognostic value in this type of cancer (Han *et al.* 1998, Aaltomaa *et al.* 1999, Musgrove *et al.* 2011). Instead, a recent study by the Knudsen Laboratory revealed that it is the expression of cyclin D1b that alters prostate cancer growth (Burd *et al.* 2006). Although cyclin D1b retains the ability to interact with AR, compared with cyclin D1a, it exhibits strongly reduced corepressor activity (Burd *et al.* 2006). As a consequence, whereas cyclin D1a inhibits AR function and AR-dependent proliferation, cyclin D1b promotes cell cycle progression and growth in prostate cancer cells (Burd *et al.* 2006).

To further delineate the mechanisms that underpin the pro-tumorigenic activity of cyclin D1b in prostate cancer, Augello *et al.* (2013) carried out microarray and gene ontology analyses to identify and characterize genes uniquely regulated by cyclin D1b. They found that a number of these cyclin D1b-regulated genes are functionally associated with altered cell migration, invasion, and differentiation. Notably, the expression of Slug (SNAI2), a member of the SNAIL family of transcriptional factors, was highly induced by cyclin D1b (Augello *et al.* 2013). Further experiments revealed that the regulation of Slug expression was achieved through the cooperation of cyclin D1b and AR, by mediating an enrichment of acetylated histones and enhanced AR occupancy at the *SNAI2* regulatory loci (Augello *et al.* 2013). Notably, Slug has been well documented to be involved in the induction of epithelial–mesenchymal transition in a variety of cancers,

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including the prostate (Medici *et al*. 2008, Emadi Baygi *et al*. 2010). Indeed, Augello *et al*. (2013) observed that knockdown of *SNAI2* by siRNA significantly suppressed the invasive capacity of prostate cancer cells. In addition, this effect was more pronounced in cells that over-expressed cyclin D1b (Augello *et al*. 2013). Taken together, these findings support that Slug is necessary and sufficient in inducing cyclin D1b-mediated pro-metastatic properties.

Interestingly, Slug has also been proposed to be a novel AR coactivator capable of enhancing AR transcriptional activities and its elevated expression was shown to facilitate AR-mediated androgen-independent cell growth (Wu *et al*. 2012). Hence, we speculate that the cooperative signaling between cyclin D1b and AR can positively feedback via Slug to enhance AR functional activity, leading to exacerbation of initial tumorigenic impacts. Most importantly, this cyclin D1b/Slug network was found to be conserved in clinical samples of CRPC (Augello *et al*. 2013). Collectively, these data provide a framework of how reactivated AR signaling works in concert with oncogenic pathways to mediate the metastatic progression of prostate cancer.

Genomic aberrations and epigenetic modifications in CRPC

The progression of prostate cancer to a castrate-resistant state is often associated with global changes in the genomic and epigenetic landscape (Seligson *et al*. 2005, Kim *et al*. 2007). Identifying the players that are involved and teasing out the detailed mechanisms used by these factors will help us to understand how genomics and epigenetics are linked to prostate cancer progression. Below, we discuss some of the recent progress in these areas in particular with respect to AR signaling and CRPC.

To gain insights into the global alterations in structural and epigenetic state of CRPC, Friedlander *et al*. (2012) generated comprehensive genome-wide maps of copy number variant regions and DNA methylation using array comparative genomic hybridization and bisulfite methylation profiling of 15 metastatic CRPC tumors respectively. Besides detecting known common copy number variations such as a gain of AR and the loss of PTEN and RB1, the authors also identified 492 other genes that were frequently amplified or deleted in CRPC. Moreover, in AR-unamplified tumors that nonetheless progressed to being castrate resistant, the authors observed a significant loss in genes previously implicated as repressors of AR activity. This observation suggests that CRPC that do not have AR amplification could continue to be driven by alterations to AR signaling, as opposed to being independent of AR pathways. Besides being a rare resource that offers insight into genes aberrant in metastatic CRPC, this study also provided a complete methylation signature of this lethal form of disease. Overall, CRPC is slightly hypermethylated compared with benign prostate tissue. Furthermore, genes that are frequently methylated or altered in copy number were mapped to three common pathways, namely androgen biosynthesis, p53, and IGF1-protein kinase B signaling pathway. Interestingly, CpG methylation was observed to occur more frequently for genes commonly deleted than for those commonly amplified. Based on this, the group speculated that copy number variation and methylation may cooperate to inhibit the expression of critical tumor suppressors, such as RB1 (Friedlander *et al*. 2012).

In addition to being misexpressed due to copy number and methylation changes, protein factors that modify the epigenetic state of the cell may also be mutated in cancer and the consequent disruption of epigenetic processes can result in aberrant gene expression programs that drive cancer progression (Esteller 2008, Sharma *et al*. 2010). In a recent landmark study characterizing the mutational landscape of CRPC, Grasso *et al*. (2012) found that the chromodomain helicase DNA binding protein 1 (CHD1) gene was frequently mutated in both localized and CRPC tumors. Although CHD1 has not been shown to physically interact with AR, the ATP-dependent chromatin-remodeling enzyme has been implicated in recruiting AR to gene promoters and the regulation of DHT-dependent gene transcription (Burkhardt *et al*. 2013). Besides CHD1, lysine (K)-specific methyltransferase 2D (MLL2), a H3K4-specific histone methyltransferase, was found to be mutated in 8.6% of prostate cancer (Grasso *et al*. 2012). Importantly, the authors showed that members of the MLL complex (including MLL2) physically interacted with endogenous AR and knockdown of the MLL complex significantly inhibited AR signaling; however, the underlying mechanism and any effect on prostate tumor growth is unclear and will require further investigation (Grasso *et al*. 2012). While it appears that the MLL complex is important in the regulation of AR-mediated transcription, further experimentations will be required to understand the specific function and significance of this complex in CRPC.

In addition to modulating the transcriptional activity and output of AR signaling to mediate oncogenesis in prostate cancer cells, epigenetic control may also contribute to castrate resistance through the deregulation of *AR* expression (Cai *et al*. 2011*b*). For example, the Balk

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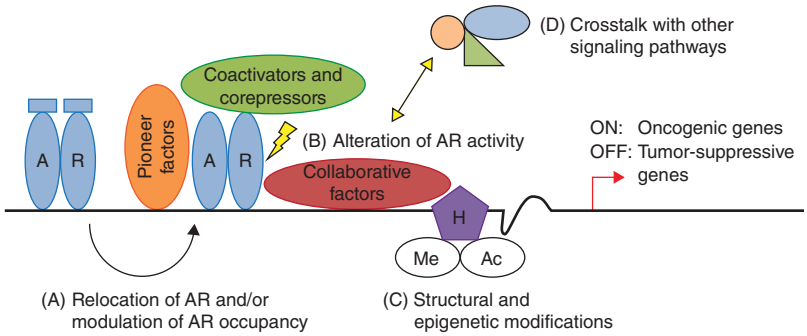

Figure 1

Transcriptional mechanisms in castrate-resistant prostate cancer (CRPC). Ligand-dependent androgen receptor (AR) is an important player in prostate development and oncogenesis. Treatment of prostate cancer with androgen deprivation therapy is effective at first, but most tumors progress to an aggressive state known as CRPC. Despite low or negligible levels of androgen in CRPC, AR signaling remains critical. A whole repertoire of AR co-regulatory factors are co-localized at AR binding sites and contribute extensively to the reactivation of AR signaling in CRPC. (A) Pioneer factors such as FoxA1 can redirect AR to selected genes and/or modulate the occupancy of AR at selected loci. (B) Coactivators and corepressors can enhance or reduce the transcriptional activity of AR respectively. (C) Factors that modify the epigenetic state of chromatin such as EZH2 can bring about altered expression of genes. (D) Other signaling cascades including PI3K/Akt can cross talk with AR signaling pathway to regulate transcription. Together, these form part of the AR co-regulatory network that turns on oncogenic genes while switching off tumor-suppressive ones, and in the process promote the progression of prostate cancer.

Laboratory demonstrated using ChIP-PCR in VCaP cells (a CRPC cell line model) that AR directly suppresses its own expression in response to androgen stimulation by binding to an enhancer region located in the second intron of the *AR* gene and recruiting the lysine-specific demethylase, LSD1, to demethylate histones H3K4me1, 2 (Cai *et al.* 2011*b*). In addition to the *AR* gene, genes involved in DNA synthesis and cell cycle progression were also repressed by agonist-stimulated AR in a similar fashion, which is consistent with the function of AR in driving normal prostate epithelium to terminal differentiation and maintaining tissue homeostasis (Cai *et al.* 2011*b*). However, in relapsed castration-resistant VCaP xenografts, the expression of multiple androgen-repressed genes, including *AR* and genes involved in DNA synthesis and cell cycle progression, were instead highly upregulated in response to androgen withdrawal (Cai *et al.* 2011*b*). Collectively, these results suggest that androgen deprivation therapy may in fact play a part in restoring AR signaling in CRPC, through the relief of AR and LSD1 repression on *AR* and other target genes.

Challenges and future perspectives

During the past decade, huge strides have been made in understanding the control of AR transcription, with valuable knowledge on processes of prostate cancer initiation and progression gained in the process. Herein, we have reviewed potential key transcriptional mechanisms that may enable cancer cells to become resistant to castration (Fig. 1). In summary, coactivators and corepressors can modulate the transcriptional activity of AR, while collaborative factors such as FoxA1 and ERG can alter the genomic occupancy of AR and modify its transcriptional output. In addition, epigenetic modifiers like EZH2 and MLL2 can influence AR transcription and target gene expression. Lastly, AR signaling can also cross talk with other signaling pathways, leading to changes in the expression of tumorigenic genes. Continued research into the transcriptional network of AR in both hormone responsive and CRPC will not only yield mechanistic insights into NR biology and pathology but also propose novel candidates of biomarkers as well as drug targets for improved prostate cancer therapy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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