

Rationale for the development of alternative forms of androgen deprivation therapy

Sangeeta Kumari^{1,*}, Dhirodatta Senapati^{1,*} and Hannelore V Heemers^{1,2,3}

¹Department of Cancer Biology, Cleveland Clinic, Cleveland, Ohio, USA

²Department of Urology, Cleveland Clinic, Cleveland, Ohio, USA

³Department of Hematology/Medical Oncology, Cleveland Clinic, Cleveland, Ohio, USA

*(S Kumari and D Senapati contributed equally to this work)

Correspondence
should be addressed
to H V Heemers
Email
heemerh@ccf.org

Abstract

With few exceptions, the almost 30,000 prostate cancer deaths annually in the United States are due to failure of androgen deprivation therapy. Androgen deprivation therapy prevents ligand-activation of the androgen receptor. Despite initial remission after androgen deprivation therapy, prostate cancer almost invariably progresses while continuing to rely on androgen receptor action. Androgen receptor's transcriptional output, which ultimately controls prostate cancer behavior, is an alternative therapeutic target, but its molecular regulation is poorly understood. Recent insights in the molecular mechanisms by which the androgen receptor controls transcription of its target genes are uncovering gene specificity as well as context-dependency. Heterogeneity in the androgen receptor's transcriptional output is reflected both in its recruitment to diverse cognate DNA binding motifs and in its preferential interaction with associated pioneering factors, other secondary transcription factors and coregulators at those sites. This variability suggests that multiple, distinct modes of androgen receptor action that regulate diverse aspects of prostate cancer biology and contribute differentially to prostate cancer's clinical progression are active simultaneously in prostate cancer cells. Recent progress in the development of peptidomimetics and small molecules, and application of Chem-Seq approaches indicate the feasibility for selective disruption of critical protein–protein and protein–DNA interactions in transcriptional complexes. Here, we review the recent literature on the different molecular mechanisms by which the androgen receptor transcriptionally controls prostate cancer progression, and we explore the potential to translate these insights into novel, more selective forms of therapies that may bypass prostate cancer's resistance to conventional androgen deprivation therapy.

Key Words

- ▶ androgen receptor
- ▶ prostate cancer
- ▶ nuclear receptor
- ▶ coregulator
- ▶ transcription
- ▶ castration
- ▶ hormonal therapy

Endocrine-Related Cancer
(2017) **24**, R275–R295

Introduction

The pivotal role of the androgen-activated androgen receptor (AR) as a driver of prostate cancer (CaP) progression has long been recognized (Denmeade & Isaacs 2002, Schmidt & Tindall 2013). The first clinical androgen

deprivation studies, published in 1941, indicated already that surgical or medical castration induces remission of, but does not cure, metastatic CaP (Huggins 1941, Huggins & Hodges 1941). Today, despite the development of an

ever increasing and more refined repertoire of drugs and treatment regimens to achieve androgen deprivation therapy (ADT) (Nguyen *et al.* 2014, Magnan *et al.* 2015, Valenca *et al.* 2015, Attard *et al.* 2016), failure of ADT is still a clinical reality that contributes directly to the ~30,000 CaP deaths in the US annually (Siegel *et al.* 2017). Aberrant re-activation of AR has been identified as the main culprit for CaP's acquired resistance to ADT. The selective pressure of ADT induces amplification, somatic mutations and ligand-independent activation of AR, stimulates intra-CaP production of AR-activating androgens, turns ADT drugs into partial AR agonists and/or modifies the activity of key AR-co-operating proteins (Heemers & Tindall 2005, 2010, Attard *et al.* 2009, Knudsen & Penning 2010, Karantanos *et al.* 2015, Li *et al.* 2015, 2016, Dai *et al.* 2017). All these mechanisms, which have been the topic of several excellent reviews (see above), lead to persistent AR action and have been shown to favor CaP cell growth under ADT in preclinical cell line and animal model systems. Whole exome and whole genome sequencing efforts on clinical castration-recurrent (CR) CaP specimens confirmed the presence of somatic alterations that contribute to AR re-activation in at least 60% of patients (Grasso *et al.* 2012, Beltran *et al.* 2013, Robinson *et al.* 2015). Targeted deep sequencing of the AR gene locus on metastatic CR-CaP samples obtained at rapid autopsy and on circulating tumor DNA from CR-CaP patient serum have isolated additional genomic structural alterations in the AR gene that give rise to androgen-independent AR forms in 30–50% of patients (Henzler *et al.* 2016, De Laere *et al.* 2017). The latter findings indicate that the actual fraction of patients whose CR-CaP harbors AR alterations is likely even higher.

Persistent AR action that controls CaP progression while CaP no longer responds to ADT represents a critical stalemate that prevents the much-needed improvements in the care and survival rates for patients suffering from CR-CaP. An alternative approach to target for therapy aberrant AR action in CR-CaP may be found by taking a fresh look at a manner by which activated AR affects the behavior of CaP cells and disease progression (Fig. 1). AR is a member of the ligand-activated nuclear receptor family of transcription factors (Heinlein & Chang 2004, Heemers & Tindall 2007). AR is activated by direct binding of androgens, which causes an inactive cytoplasmic AR to undergo conformational changes, alter its association with chaperones and translocate to the nucleus. Inside the nucleus, ligand-bound AR binds to DNA recognition motifs known as androgen response elements (AREs) and

recruits from a large array of coregulators and cofactors to control transcription of ARE-driven AR target genes. For more than 7 decades, the main approach to prevent AR activation, and the current mainstay of ADT, has been to deprive AR of its ligand (Denmeade & Isaacs 2002, Schmidt & Tindall 2013). This has been accomplished by preventing the production from the most bioactive androgen dihydrotestosterone (DHT) from precursors that are derived from gonads or adrenals or are produced intra-prostatically, or by administration of anti-androgens that compete with DHT for AR binding. Other targets in the pathways leading to aberrant AR activation such as interference with the chaperone proteins clusterin or Sigma-1 (Yamamoto *et al.* 2015, Thomas *et al.* 2017), or with the signaling cascade by which cytokines or growth factors activate AR activity (Culig 2004), to name but a few, have been explored also for therapeutic intervention. Parallel efforts have focused on targeting for therapy AR moieties other than its ligand binding domain, such as its constitutively active N-terminal transactivation domain (Andersen *et al.* 2010, Myung *et al.* 2013) or its DNA binding domain (Dalal *et al.* 2014). The latter approaches have either not been tested yet in clinical trials or the results of such trials are not yet available.

Meanwhile, the technological advances that led to the genomic analyses of clinical CaP specimens have allowed also to map and characterize AREs, AR-dependent gene transcription, and the contribution of AR-associated proteins to AR target gene transcription in preclinical CaP models and in clinical specimens. The renewed insights in genome-wide DNA AR binding sites and patterns, and the dependence of AR's transcriptional output on other transcriptional regulators indicate that AR's regulation of target gene transcription does not follow a uniform pattern. The goal of this review is to summarize the knowledge that has been gained in terms of the distinct molecular mechanisms by which AR target gene transcription occurs, and to explore whether this knowledge can be exploited to develop novel therapies that block specifically the action of androgen-activated AR that controls aggressive CaP cell behavior.

Diversity in AR–DNA interactions

Many efforts have been directed toward resolving the manner in which ligand-activated AR interacts with AREs. Initially, studies focused on distinguishing between the mechanisms by which AR and related nuclear receptors, such as the glucocorticoid receptor (GR), which are

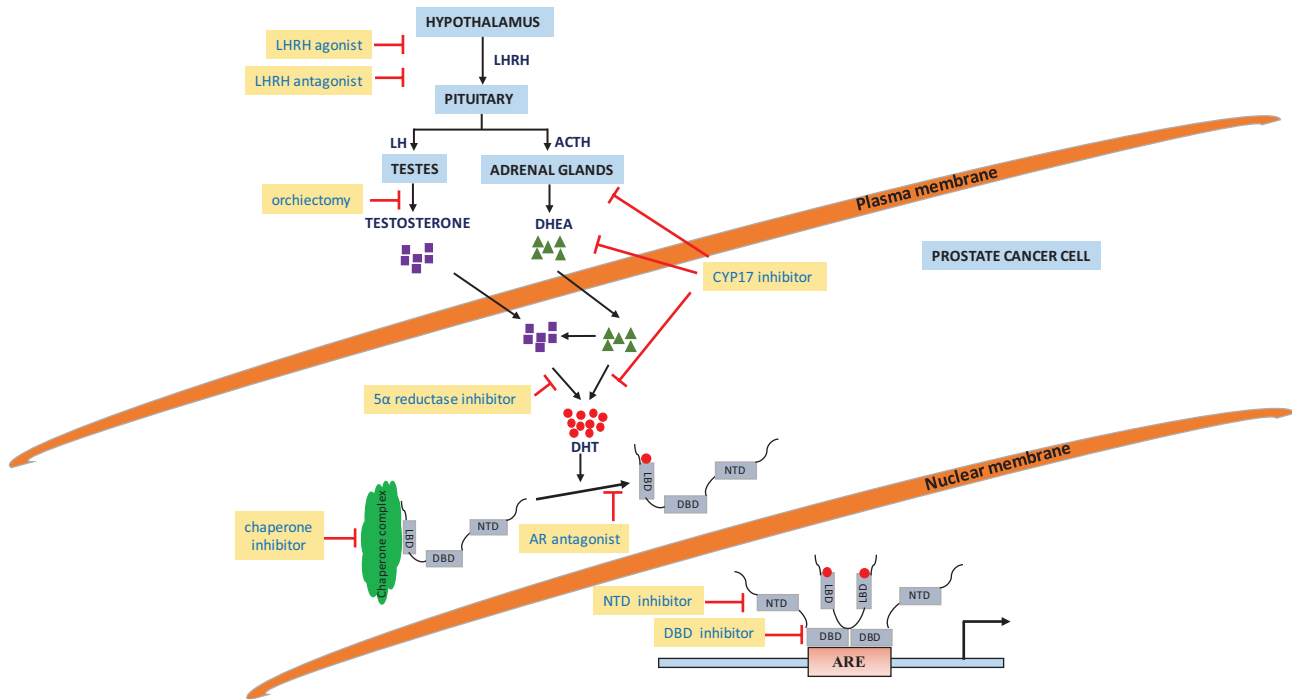


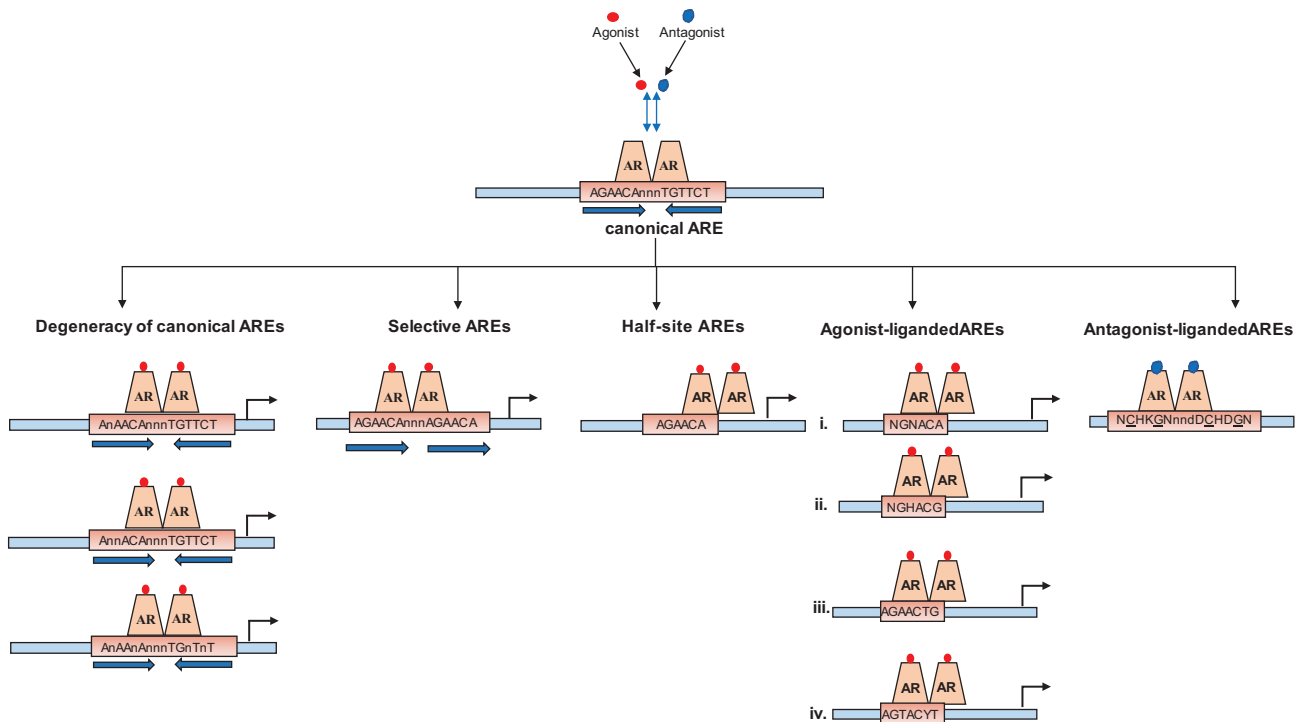
Figure 1

Basic mechanism of AR-mediated transcription. The hypothalamus produces LHRH which induces the production of LH and ACTH by the anterior pituitary. LH and ACTH stimulate secretion of testosterone and DHEA by the testes and adrenal glands, respectively. Testosterone and DHEA are converted into the bioactive androgen DHT in target cells. DHT binds to inactive AR in the cytoplasm, triggering a conformational change and nuclear translocation of AR. Inside the nucleus, DHT-bound AR homodimerizes and binds to AREs to drive the expression of AR target genes. LHRH, luteinizing hormone-releasing hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; AR, androgen receptor; NTD, N-terminal domain; LBD, ligand binding domain; DBD, DNA binding domain; ARE, androgen response element. Red lines mark current therapeutic targets to inhibit AR signaling.

recruited to highly similar DNA motifs using structurally similar protein domains, bind selectively to their respective cognate genomic binding site. Although still far from understood completely, the current consensus is that AR binds as a dimer in a head-to-head conformation to inverted repeats of a 5'-AGAACA-3' hexamer that are separated by 3 bases (Claessens *et al.* 2017). We will refer to this 15-mer sequence as the canonical ARE motif. Domains other than AR's DNA binding domain, for instance its N-terminal domain, have been recognized to exert allosteric effects on the interaction between AR and ARE (Callewaert *et al.* 2003, Tadokoro-Cuccaro *et al.* 2014).

Today's insights in the AR cistrome are derived from an array of ChIP-chip, ChIP-Seq and, more recently, ChIP-exo studies on diverse CaP model systems after treatment with different ligands for variable durations (Horie-Inoue & Inoue 2013, Jin *et al.* 2013). Because of these efforts, thousands of AR binding sites, which contain AREs and are located preferentially in enhancer and intergenic regions, have been isolated. Owing to these data, evidence is emerging that even within the same cell AR can bind different classes of AREs, only a minority of which conform

to the canonical ARE motif (Fig. 2, discussed below). Diversity in the sequence composition of the AREs that regulate AR target gene expression should perhaps not be surprising. After all, even very early after the discovery of the first AREs in genes encoding for instance secretory component (SC), sex limiting protein (SLP) or prostate-specific antigen (PSA), the existence of 2 classes of AREs was proposed. In this model, some AREs were bound by AR alone (so-called selective AREs), whereas others could be recognized by both AR and GR (classical AREs) (Verrijdt *et al.* 2006). It is now clear that the selective modes of AR-ARE interaction are not due to different dimerization patterns of AR at AREs (head-to-head vs head-to-tail (Shaffer *et al.* 2004)) as originally thought. Rather, recent ChIP-Seq data have clarified that AR binds to selective AREs because of less stringent sequence requirements for the 3' hexamer (Sahu *et al.* 2014). Recent bioinformatics-guided modeling of the ARE motifs derived from similar high-throughput studies indicated that only a minority of the AREs fit perfectly the consensus AGAACAnnnTGGTCT 15-mer motif (Wilson *et al.* 2016). The majority of AREs corresponded to 'imperfect' AREs

**Figure 2**

Schematic representation of AREs derived from ChIP-Seq and ChIP-exo analyses. Top panel, consensus sequence for a canonical ARE, which reflects the ideal 15-mer motif of two hexamers arranged in inverted repeat with a 3-mer spacer. Bottom panels, deviations from this conventional AREs, which include (from left to right) degenerate AREs in which mismatches of 1 or more basepairs from the consensus canonical ARE motif occur, selective AREs which are composed of direct repeat of 6-mer half-sites, 6-mer half-site ARE sequences found to be enriched in AR binding sites derived from ChIP-based approaches, 4 types of ChIP-exo protected agonist-liganded AREs and ChIP-exo protected antagonist-liganded AREs. Note that, until proven conclusively otherwise, AR is assumed to bind as a dimer to all ARE versions.

in which mismatches of 1 or more basepairs from the consensus motif occur. Consistent with other reports (see for instance (Massie *et al.* 2007, Wang *et al.* 2007)), for a significant number of AR binding sites only one ARE half-site that corresponds to a perfect hexamer was present. Increased levels of degeneracy of the ARE have been linked with decreased level of transcriptional output of those sites (Wilson *et al.* 2016). As technology became more sensitive and allowed for higher resolution scanning of the ARE motif and its surrounding sequence, more details could be derived on the genomic regions occupied by AR. A recent ChIP-exo study that more precisely mapped the boundaries of AR-occupied DNA, has reported 4 different classes of agonist-bound AR binding elements based on the sequence composition of the ARE (hexamer) and border patterns that were protected from exonuclease digestion (Chen *et al.* 2015) (Fig. 2). Strikingly, the latter study also identified a previously unrecognized type of motif, 5'-NCHKGNnndDCHDGN-3', to which antagonist- but not agonist-liganded AR is recruited. Some of these antagonist-specific motifs could be occupied by bicalutamide- as well as enzalutamide-liganded AR. Others

were bound only by bicalutamide- or enzalutamide-liganded AR, indicating specificity of some of these sites for specific antagonist-AR interactions.

Recently, versions of AR that lack its ligand binding domain but retain AR's DNA binding properties have been recognized to emerge under ADT, most often in the presence of full-length AR. More than 20 AR variants that arise either via alternative splicing or via genomic rearrangements in the AR gene locus have been isolated (Dehm *et al.* 2008, Hu *et al.* 2011, Henzler *et al.* 2016). These variants, the best studied of which are ARV-7 and ARv567es, have been shown to facilitate nuclear localization of full-length AR under ADT and to form homodimers as well as heterodimers with other AR variants or with full-length AR (Cao *et al.* 2014, Xu *et al.* 2015, Zhan *et al.* 2017). Whether these AR variants occupy the same AR binding sites or AREs as full-length AR is an important question, which has proven difficult to answer. Co-occupancy of canonical AR target genes by ARV-7 and full-length AR in a mutually dependent manner has been observed, whereas ARV-7 homodimers but not full-length AR were recruited to other AR variant-specific

genes (Cao *et al.* 2014). A ChIP-Seq study in cells that were engineered to express either full-length AR or ARv567es indicated that ARv567es recognizes the same target genes and canonical AREs as full-length AR, but with lower affinity (Chan *et al.* 2015). Moving forward, the use of CaP model systems in which expression levels of different AR species can be controlled tightly will help resolve their precise DNA binding patterns and the degree to which these patterns overlap.

In addition to variability in the sequence of the AREs that are present, diversity has been observed also in the DNA motifs adjacent to AREs that correspond to binding sites for other transcription factors. Soon after the identification of the same, first AREs that are discussed above, it became obvious that other transcription factors contribute differentially to androgen regulation of reporter gene activity under their control (Scarlett & Robins 1995, Zhang *et al.* 1997, Haelens *et al.* 1999). At that time, the concept of the Androgen Response Unit was proposed, which entailed that AREs are embedded in genomic domains containing also binding sites for other transcription factors that help to fine-tune the androgen response in a gene-specific manner (Celis *et al.* 1993). Several of these factors will be discussed in more detail in the following section. These initial indications have been confirmed in ChIP-based approaches that extended knowledge beyond that obtained for a few known AR target genes (e.g. Massie *et al.* 2007, Wang *et al.* 2007, 2009). Selective enrichment of binding motifs for other transactivating factors suggest that AR's binding partners at a given AREs are different, and that molecular regulation of AR's transcriptional output at different AREs varies. The relevance of such interactions is underscored further following the recent characterization of the AR cistrome at different phases of the CaP cell cycle progression (McNair *et al.* 2017). Previous AR ChIP-chip, ChIP-Seq or ChIP-exo datasets were derived from cells that were androgen deprived prior to androgen stimulation, which means from cells that were stuck in G0 phase. AR ChIP-sequencing on cells that were arrested in specific stage of the cell cycle has identified the full complement of AR binding sites in mitotically active cells. This work has uncovered a large set of previously unknown AR binding sites. Some sites were present throughout cell cycle progression, and overlapped with the already isolated AR cistrome. Others, however, were specific for select stages of the cell cycle. Motif analyses of the specific AR binding sequences derived from each step of the cell cycle revealed strikingly selective enrichment for recognition motifs for

other transcription factors. These data suggested that distinct interactions between AR and other transcriptional regulators control androgen-dependent progression through different stages of cell cycle progression.

Specific protein-protein interactions may contribute also to the differences in AR binding site patterns that are found between clinical CaP specimens and (synchronized) cell lines, and among prostate (cancer) samples obtained at different stages of disease progression. A shift in the AR cistrome between normal prostate tissues and localized ADT-naïve CaP specimens is accompanied by differential enrichment for binding sites for FoxA1 and HoxB13 in CaP-associated AR binding sites (Pomerantz *et al.* 2015). Overexpression of either of these 2 transcription factors in AR-expressing normal prostate epithelial cells induced neoplastic transformation and a 'benign-to-malignant' shift in the AR cistrome. In CR-CaP, a novel set of AR binding sites emerges that is not present in ADT-naïve CaP or in CaP cell line models. This CR-specific set of AR binding regions was enriched in binding motifs for transcription factors such as STATs and IRFs (Sharma *et al.* 2013). The relevance of specific AR-transcription factor interactions for the composition of the AR cistrome was underscored further in studies in which expression of the transcription factor FoxA1 was silenced. After silencing, some of the AR binding sites that were present before the loss of FoxA1 were gone, some were maintained, and a large number of novel AR binding sites were gained. Motif analysis of these regions indicated that gained AR binding sites harbor a classical 15-mer ARE consensus motif whereas the sites that were lost tended to be composed of a half-site ARE and a FoxA1 binding motif (Wang *et al.* 2011a). These data indicate that AR-interacting proteins may restrict AR binding to some genomic sites or direct AR binding to others. Similar behavior has been noted also for the AR-associated transcription factor GATA2 (Zhao *et al.* 2016).

Transcriptional regulation of gene expression results from an interplay between DNA sequence, transcription factor action, chromatin accessibility and nucleosome assembly. It is, therefore, important to note that sequence analyses on data from nucleosome (re)positioning and DNase I hypersensitivity assays after androgen treatment of CaP cells support diversity in AR binding site composition and AR binding partners. Examination of the position of H3K4me2 marks, which are associated with transcriptionally active enhancers, have shown that androgen treatment leads to dismissal of a central nucleosome over the AR binding site and repositioning of flanking nucleosomes (He *et al.* 2010). These nucleosome

relocation patterns predicted the presence of binding sites for AR and AR-associated transcription factors such as FoxA1 and Oct1. Furthermore, a direct comparison of the DNA regions obtained by DNase I protection assays and AR ChIP sequencing data from androgen-treated CaP cells revealed 3 different footprinting patterns that overlap with AR binding sites (Tewari *et al.* 2012). Two of these patterns contain an ARE half-site and binding motifs for Fox family members, whereas the third harbors full-length AREs and are specifically enriched for motifs recognized by NF1C.

Taken together, literature indicates that AR's recruitment to a diversity of ARE motifs where it interacts selectively with other transcription factors that bind their own recognition motif leads to heterogeneity in AR target gene transcription. In addition, AR-interacting transcription factors may control AR's access to genomic AR binding sites.

Heterogeneity in the interaction between AR and its associated transcriptional regulators at AREs

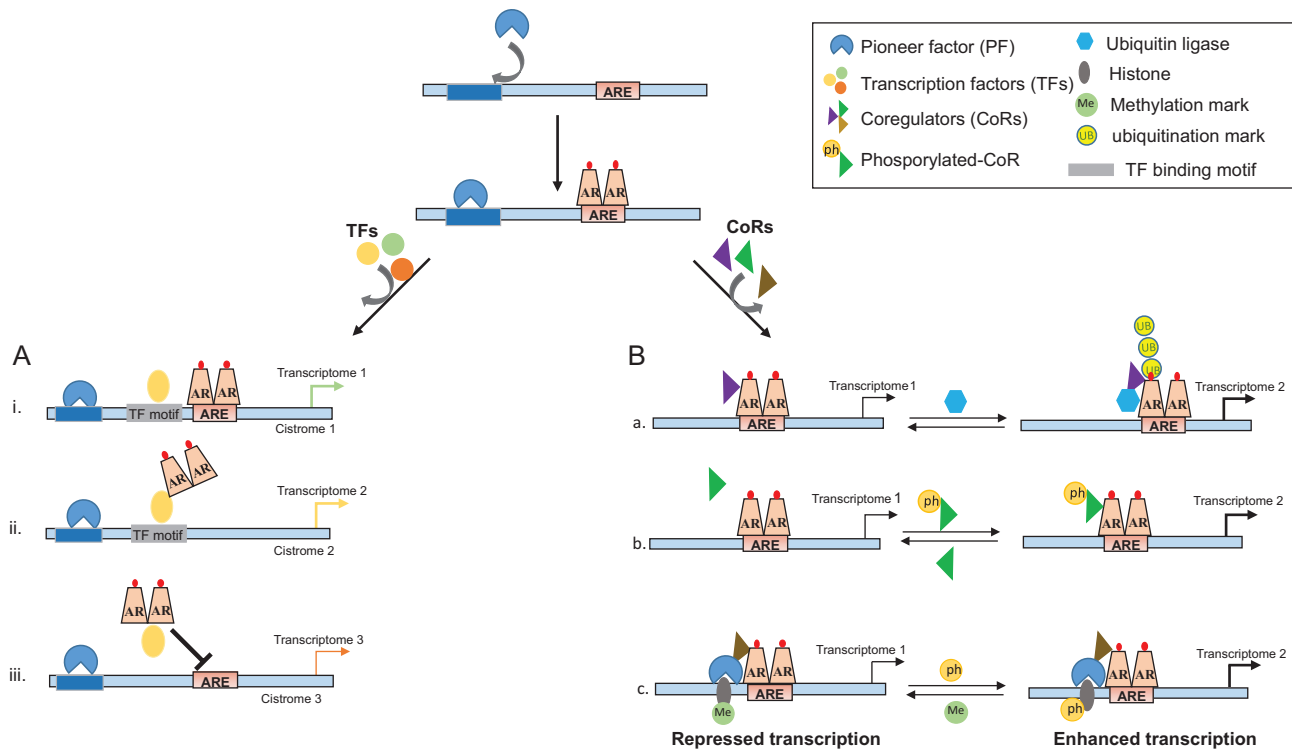
DNA-bound AR executes transcription of its target genes via interaction with a large number of functionally diverse proteins (Heemers & Tindall 2007, Gottlieb *et al.* 2012, DePriest *et al.* 2016). Not taken into account the general transcriptional machinery, these proteins fall into 2 major classes: transcription factors that bind their own consensus binding motifs nearby AREs and coregulators that associate with ARE-bound AR without binding DNA themselves. More than 300 proteins that belong to the latter 2 categories have been reported to contribute directly to AR-mediated transcription. In all likelihood that number is a significant underestimation considering the hundreds of proteins that have been identified as previously unrecognized coregulators via IP-mass spectrometry approaches, and the novel interaction modes among such proteins (Malovannaya *et al.* 2011). Recent mapping of the genome-wide binding sites of some of these AR-associated proteins indicate variability in recruitment patterns to different AREs. Such diversity could signify the formation of differentially composed protein-protein complexes at AREs and thus selective control by these complexes over specific biological processes in CaP cells. This possibility is in line with findings from gene expression studies in which key AR-associated regulators were silenced or inhibited, which affected androgen regulation of subfractions only

of the AR transcriptome. In this section, we provide an overview of the mechanisms by which pioneer transcription factors, other transcription factors, and coregulators may contribute to a selective output from the AR transcriptional complex (Fig. 3).

Pioneer transcription factors

The isolation of the AR cistrome via ChIP-based approaches has provided insights also in the manner in which AR is recruited to unoccupied AREs in a largely inaccessible chromatin environment after androgen stimulation. As for other transcription factors, AR recruitment is facilitated by, and dependent on, pioneer factors. These factors are able to bind to compact chromatin before AR does, open up the local chromatin environment, and make it competent for the AR to execute the transcription of its target genes (Zaret & Carroll 2011, Jozwik & Carroll 2012).

The first ChIP-chip study that defined the AR binding sites on chromosomes 21 and 22 reported co-occurrence of AREs (half-sites) with 3 significantly enriched DNA motifs, namely those to which Forkhead, GATA, and Oct transcription factors are recruited (Wang *et al.* 2007). These sites were subsequently shown to be occupied preferentially by FoxA1, GATA2 and Oct1, respectively. The 3 factors were recruited in various combinations, independently or simultaneously, to 80% of the AR binding sites tested, which indicated already gene selectivity. Strikingly, significant binding of each of the 3 factors to the AR binding regions was observed already in the absence of androgens, which led to their being labeled as potential pioneer factors that facilitate subsequent AR-dependent transcription. None-the-less, androgen treatment increased also the specific factor binding at specific sites. Interactions of FoxA1, GATA2 and Oct1 with AR at a few binding sites that were selected for further study (those present in *PSA* and *TMPRSS2* genes) depended on their respective functional cis-acting motifs. AR formed independent complexes with each factor, and interacted with GATA2 and Oct1 in a hormone-dependent manner while its interaction with FoxA1 was not affected by androgens. Recruitment of AR to these select ARE-containing enhancers was significantly decreased following transfection with siRNAs targeting FoxA1 or GATA2, both in the absence of ligand and, to a greater extent, in the presence of hormone. In contrast, silencing of Oct1 did not affect AR binding on the *PSA* and *TMPRSS2* enhancers in the absence and presence of hormone. Oct1 recruitment to the *PSA* and *TMPRSS2* enhancers was

**Figure 3**

Schematic depiction of some of the mechanisms by which pioneer transcription factors, other transcription factors and coregulators selectively contribute to AR-dependent transcription. Top panel, pioneer factor opens up the chromatin environment to facilitate AR–ARE interactions and subsequent recruitment of other components of the AR transcriptional complex. Recruited components include other transcription factors (TFs) (A) and coregulators (B). (A) Schematic representation of the manner in which a single TF (e.g. HoxB13) either increases (i and ii) or decreases (iii) androgen regulation of select AR target genes. (B) Schematic representation of the mechanisms which coregulators selectively control portions of AR's transcriptional output. Coregulators can enhance the transcriptional activity of AR by degrading a transcriptionally inactive pool of AR (Siah2, a), bind to AR only when it has undergone a specific post-translational modification (ph-Ezh2, b) and (c) bind at specific AREs guided by histone modification patterns to act as coactivator or corepressor (LSD-1, c).

similarly attenuated when GATA2 was silenced, whereas GATA2's presence was not affected by Oct1 silencing. These data suggested that GATA2, FoxA1 and Oct1 play necessary roles both in the basal recruitment of AR to chromatin and in their recruitment following androgen stimulation. Interestingly, these data indicated also that GATA2 and Oct1 act at distinct steps in AR signaling, with GATA2 acting upstream of Oct1 recruitment.

Independent ChIP-Seq studies to define the genome-wide recruitment pattern of these factors have confirmed and expanded on the initial findings for candidate genes. It is now evident that more than 50, 25 and 10% of AR recruitment sites overlap with binding sites for FoxA1, GATA2 and Oct1, respectively (Sahu *et al.* 2011, 2012, He *et al.* 2014, Wu *et al.* 2014, Obinata *et al.* 2016, Zhao *et al.* 2016). These numbers are rough estimates only as individual studies used variable treatment durations, different AR agonists or were done in different cell lines. Some ARE-harboring genes contained binding peaks for

the 3 factors, whereas others harbored only recruitment sites for one or two factors. Gene selectivity of these binding patterns was reflected also in corresponding gene expression studies, which showed that androgen response of portions of the AR transcriptome only were altered after silencing of each factor individually (Sahu *et al.* 2011, He *et al.* 2014, Wu *et al.* 2014, Obinata *et al.* 2016, Zhao *et al.* 2016).

The role for pioneer factors in AR-dependent transcription is sustained, and it evolves, during CaP progression. Direct comparisons between isogenic cell lines that represent ADT-naïve CaP or CR-CaP have shown higher occupancy of FoxA1 and GATA2 at representative CR-specific AR target genes such as UBE2C (Wang *et al.* 2009). Similarly, loss of FoxA1 and GATA2 reduced expression of these CR-CaP-specific AR target genes only in CR-CaP cells, even though the expression levels of FoxA1 and GATA2 were not elevated in these cells. Differential levels of gene occupancy for these factors correlated with

differences in gene expression patterns also in clinical CaP specimens obtained before and after ADT and may, thus, contribute to AR transcriptome differences between ADT-naïve and CR-CaP.

Consistent with the findings for FoxA1 that are mentioned above, the composition of the AR cistrome is impacted also by the action of GATA2 and Oct1. Silencing of GATA2 and Oct1 led to loss of a fraction of AR binding sites while other sites were maintained. New AR binding sites were gained also (Obinata *et al.* 2016, Zhao *et al.* 2016). However, the number of these novel sites was significantly lower than that gained after loss of FoxA1 expression, and the gained sites did not show enrichment of 15mer ARE motifs. Supporting the notion of a higher order in the action of these transcription factors, silencing of FoxA1 led to redistribution of the GATA2 cistrome, whereas loss of GATA2 did not impact on the location of the FoxA1 binding sites (Zhao *et al.* 2016).

Taken together, these findings indicate that FoxA1 and GATA2, and perhaps to a lesser extent Oct1, function as pioneer transcription factors for AR, in a gene- and/or location-specific manner. Hierarchy exists in the action of these proteins for generation of the AR cistrome wherein FoxA1 can program also GATA2's pioneering function and GATA2 acts upstream of Oct1 (Wang *et al.* 2007, Zhao *et al.* 2016). Evidence is emerging also for additional, non-pioneering roles for these 3 factors in AR-dependent transcription, for instance control over AR expression levels (He *et al.* 2014) and restricting AR access from select AREs (Sahu *et al.* 2011, Wang *et al.* 2011a).

Non-pioneer transcription factors

Analyses similar to those that isolated the FKHD, GATA and Oct consensus motifs in AR binding sites have been done for most, if not all, ChIP-based genomic AR mapping efforts. These studies have recognized that consensus binding motifs for transcription factors that do not fit a pioneering role are also significantly enriched near the AR cistrome. Examples include recognition sites for CREB and Nkx3.1 (Massie *et al.* 2007, He *et al.* 2010, Tan *et al.* 2012). As indicated above, several of these transcription factors, for instance HoxB13 and IRFs (Sharma *et al.* 2013, Pomerantz *et al.* 2015), may contribute in a stage-specific manner to AR cistrome programming that occurs during clinical CaP progression. Others, such as ETS transcription factors, have been implicated in aggressive CaP cell behavior in preclinical model systems (Klezovitch *et al.* 2008, Tomlins *et al.* 2008, Wang *et al.* 2008a). Although

androgen-dependent cistromes for all the transcription factors for which a motif has been isolated near AR binding sites are not yet available, the DNA binding patterns for the factors that have been examined do suggest contribution to a subset of AR target genes and the control over distinct CaP cell biology. Here, we highlight a few of these factors, the manner in which they interact with DNA-bound AR, and their role in specific AR target gene expression patterns that are relevant to CaP progression and survival.

ETS transcription factor family members A pioneering AR ChIP-chip study on 24,275 promoter-only gene regions yielded 1532 promoter domains to which AR bound after androgen stimulation of LNCaP cells (Massie *et al.* 2007). When analyzing the corresponding DNA sequences, the canonical 15-mer ARE was found in less than 10% of AR binding sites. Instead, the most frequently occurring motif was that of an ARE half-site, which was present in almost 80% of promoter regions. This motif was closely followed in frequency by a consensus binding sequence for the avian erythroblastosis virus E26 homolog (ETS) family of transcription factors, which was found in 70% of AR binding sites. Validation experiments confirmed that the ETS1 family member can bind to AREs. ETS1 was present also in AR immunocomplexes and selectively contributed to androgen regulation of ARE-driven genes that contain also the 6-mer ETS motif, but not genes that do not harbor such a motif. Strikingly, ETS1 was recruited to AR binding sites in an androgen-dependent manner, which did not fit the behavior of a pioneer factor. These findings thus uncovered a novel, selective role for ETS family members in AR-dependent transcription.

ETS1 belongs to the same family of transcription factors as ERG. ERG's function in CaP has become a hot topic since the identification of the TMPRSS2-ERG gene fusion, which has been linked to CaP cell invasion in preclinical models and to induction of CaP precursor-like lesions in mice (Tomlins *et al.* 2005, 2008, Klezovitch *et al.* 2008, Wang *et al.* 2008a).

The TMPRSS2-ERG gene fusion is present in about half of localized CaP cases, which makes it the most frequent gene-specific alteration in organ-confined treatment-naïve CaP (Cancer Genome Atlas Research Network. Electronic address scmo & Cancer Genome Atlas Research Network 2015). Although these gene fusions have been associated with the early onset of CaP (Weischenfeldt *et al.* 2013, Steurer *et al.* 2014), their clinical utility as a prognostic or

predictive biomarker for CaP progression is still unclear. Many studies have evaluated their role in patients' outcome after radical prostatectomy, risk for biochemical recurrence, response to ADT and lethal CaP development (reviewed in [Bostrom et al. 2015](#)) and have reached different conclusions. The reason for the discrepancies between these studies is not entirely clear, but is likely related at least in part to differences in TMPRSS2-ERG fusion status among and within intraprostatic foci of localized CaP ([Svensson et al. 2011](#), [Boutros et al. 2015](#), [Wei et al. 2016](#)). Inconsistent association between TMPRSS2-ERG status and post-operative risk for CaP progression may, thus, be due to inadequate tissue sampling.

The ERG and AR cistromes have been defined in VCaP cells, which harbor the TMPRSS2-ERG fusion ([Yu et al. 2010](#)). ChIP-Seq analysis was done, which allowed to assess also occupancy of enhancer and intergenic regions. In this work, the canonical 15-mer ARE was isolated as the most enriched motif in AR binding peaks and the ETS family motif ranked as the second enriched motif. Notably, molecular concept mapping of AR-bound gene sets also reported an enrichment in ERG-regulated genes. 44% of the AR-bound gene regions in VCaP cells overlapped with ERG binding peaks, and, strikingly, AR and ERG ChIP-Seq analysis of an ERG-TMPRSS2 positive clinical CaP specimen also identified a 44% overlap in AR- and ERG-enriched genomic regions. In addition, the biology of genes bound by AR and ERG *in vivo* reflected AR and ERG function in *in vitro* LNCaP and VCaP cell lines. Mechanistically, ERG was found to repress AR function in part by binding the promoter of the AR gene and decreasing AR expression. ERG overexpression correlated with repression of AR target genes *in vivo* and decreased androgen regulation of a lineage-specific differentiation program. Gradual overexpression of ERG in ERG-negative LNCaP cells also gradually increased the ERG binding but decreased AR binding at gene-specific loci. Conversely, ChIP-Seq on VCaP cells in which ERG had been silenced led to 24% decrease in ERG binding sites and a 26% increase in AR binding sites. This molecular regulation of the AR-dependent cistrome and transcriptome by ERG indicated selective effects on AR action that inhibit specifically AR-dependent pro-differentiation potentiating pathways and result in CaP dedifferentiation.

In addition to ETS1 and ERG, other ETS family members such as ETV1 and GABP α have been shown also to modulate AR signaling in CaP cells ([Baena et al. 2013](#), [Sharma et al. 2014](#)). Analyses on the cistromes of these 4 ETS factors have indicated that both specificity and

synergy may exist in their contribution to AR action and CaP biology ([Sharma et al. 2014](#)).

HoxB13 HoxB13 is a member of the homeodomain family of sequence-specific transcription factors. During embryogenesis, HoxB13 is expressed prominently in the urogenital sinus and plays a critical role in the development of the prostate ([Zeltser et al. 1996](#), [Huang et al. 2007](#)). HoxB13 was identified as an AR-interacting protein via a T7 phage display ([Norris et al. 2009b](#)). Although reported before to repress androgen-dependent reporter gene activity ([Jung et al. 2004](#)), HoxB13 was shown to selectively induce or repress transcription of androgen-responsive genes ([Norris et al. 2009a](#)). These differential effects were linked to its ability to increase or decrease recruitment of AR and AR-associated coregulators to genomic AR and/or HoxB13 binding sites in the affected genes. Specifically, at genes for which HoxB13 acts as a transcriptional repressor, loss of HoxB13 increased binding of AR and coregulators such as TRAP220, p300 and SRC1. Conversely, at genes for which HoxB13 acts as a transcriptional activator, silencing of HoxB13 led to a decrease in both AR and coregulatory occupancy, or maintenance of AR but loss of AR-associated coregulators. These insights were derived from studying a set of 6 ARE-driven genes for which AR and HoxB13 binding sites and recruitment modes have been thoroughly characterized ([Norris et al. 2009a](#)). Elegant studies in which AR and/or HoxB13 DNA binding motifs were mutated or in which the DNA binding domain of AR or HoxB13 underwent mutations to prevent DNA binding uncovered 3 molecular mechanisms of HoxB13 action at these AR-recruiting genes. For AR target genes for which HoxB13 acts as an activator, 2 models were identified. In genes for which the collaborative model applies, DNA binding sites for AR and HoxB13 were present and both AR and HoxB13 bound. In the alternative, tethering model, genes did not harbor canonical AREs but AR was recruited via HoxB13 that was bound to its own consensus motif. A third repressor model applied to genes for which HoxB13 acts as a repressor. Genes whose androgen regulation follows the latter mode of HoxB13 regulation contained AREs but not HoxB13 binding motifs, and HoxB13's interaction with the AR DBD prevented AR from binding and activating gene transcription via AREs. This differential molecular contribution of HoxB13 to AR target gene expression likely underlied observed differential effects of HoxB13 on androgen-responsive CaP cellular processes. Silencing of HoxB13 for instance decreased androgen regulation of cell

proliferation but increased aspects of cell differentiation, for instance lipogenesis (Norris *et al.* 2009a).

The potential relevance of these findings becomes more obvious taking into account the contribution and defining role of HoxB13 in establishing the clinical AR CaP cistrome (Pomerantz *et al.* 2015). HoxB13 binding sites, along with those for FoxA1, are enriched significantly in AR binding sites isolated in clinical localized CaP compared to those found in benign prostate samples. Co-expression of FoxA1 and HoxB13 in an immortalized prostate epithelial cell line caused the AR cistrome to resemble that of CaP and conferred neoplastic transformation to the cells. ChIP-Seq studies on clinical CaP specimens and cell lines confirmed extensive and selective overlap between CaP-associated but not normal prostate-associated AR binding peaks and HoxB13 peaks. Collectively, these data put HoxB13, and the molecular mechanisms by which it controls select biological processes, forward as key determinants of lineage-specific CaP-relevant AR action.

NANOG The pluripotent transcription factor NANOG has been shown to be overexpressed gradually during the progression from benign prostate tissue to lethal CaP, and to promote CR progression in CaP models (Jeter *et al.* 2009, 2011, 2016). In CaP cells, NANOG is predominantly derived from a retrogene (NANOG version known as NP8), although it can be expressed also from the NANOG1 locus (version referred to as NANOG1). We will use the term NANOG to refer to both forms as they behave very similarly in the studies outlined below. ChIP-Seq studies were performed to derive the mechanistic underpinnings of the dependency of CR-CaP on NANOG for growth and regeneration (Jeter *et al.* 2016). ChIP-Seq was done in LNCaP cells that inducibly overexpressed NANOG, as the low levels of endogenously expressed NANOG protein impeded generation of reliable data. The NANOG cistrome in these cells showed little (<10%) overlap with that obtained from embryonic stem cells, indicating the existence of a CaP-specific NANOG cistrome. Motif analyses of NANOG binding peaks indicated significant enrichment for binding sites for FoxA1 and Nkx3.1, both factors that are known to interact with DNA-bound AR (Wang *et al.* 2007, Tan *et al.* 2012). Comparison of NANOG's DNA occupancy with that of AR demonstrated coordinated binding patterns (up to 70% co-occupancy) and revealed that NANOG occupancy under ADT-naïve conditions most closely resembled that of the CR AR cistrome, suggesting that NANOG reprograms AR cistrome to facilitate CR recurrence. Co-localization and functional interaction between

NANOG, AR as well as FoxA1 was confirmed using an array of protein–protein interaction assays. RNA-Seq analyses done at different time points in LNCaP cells that were either androgen-stimulated or -deprived identified distinct clusters of genes for which androgen regulation was activated or repressed, respectively, following overexpressing of NANOG (Jeter *et al.* 2016). For instance, clusters of genes were repressed by NANOG under both androgen-supplemented and androgen-deprived conditions. These genes correlated with AR-regulated cell differentiation and sensitivity to ADT, and were highly enriched for co-occupancy of AR, FoxA1 and NANOG. These expression data suggested that NANOG suppresses pro-differentiation genes and supports development of CR-CaP. Remarkably, genes belonging to these clusters were able to stratify patients into low- vs high-risk groups and predicted favorable patient survival. Two other sets of genes were upregulated early in androgen-supplemented conditions, and their expression went down at later time points and was downregulated also under androgen-deprived conditions. These expression patterns suggested that these genes are rapidly induced by NANOG to facilitate CR-CaP progression and require ligand binding for their sustained expression. These genes were less enriched for NANOG, AR and FoxA1 co-occupancy, although 40–50% of peaks occupied by NANOG still recruited also AR and/or FoxA1. The molecular basis for the functional interplay between AR and NANOG at select AR target genes that facilitates resistance to ADT and CR-CaP progression has yet to be elucidated. It is tempting to speculate that both competitive and co-operative models of AR–NANOG interaction, similar to those described for HoxB13's role in AR action, may be at play here and could involve also the actions of FoxA1 and/or NKX31.

The reports discussed here indicate differential co-operation between AR and non-pioneering transcription factors, which translates into diverse composition of the AR cistrome and AR-dependent transcriptome. The examples highlighted here relate to transcription factors with functions in lineage-specificity, pluripotency or cell differentiation. It is likely that AR's interaction with other factors controls other gene sets that regulate other aspects of androgen-dependent CaP cell biology. Ultimately, these select AR-transcription factor interactions differentially impact on CaP behavior and progression.

Coregulators

AR's transcriptional output is modulated further by a continuously growing number of coregulators (currently

$n > 274$) (Heemers & Tindall 2007, DePriest et al. 2016). Coregulators are recruited by AR to AREs, but do not bind DNA directly. Several AR-associated coregulators are overexpressed in CR-CaP, i.e. in the presence of AR that has been 're-activated' under ADT, compared to localized CaP (Table 1) (Gregory et al. 2001, Varambally et al. 2002, Halkidou et al. 2003, Comuzzi et al. 2004, Agoulnik et al. 2006, Logan et al. 2006, Peng et al. 2008, Karpf et al. 2009, Balasubramaniam et al. 2013, Goodwin et al. 2013, Puhr et al. 2014, Schrecengost et al. 2014, Malik et al. 2015, Groner et al. 2016). While AR-associated coregulators do regulate AR's transcriptional output, and some have been claimed to be specific for AR at the time of isolation (Yeh & Chang 1996), it is now clear that their actions are generally not restricted to AR but regulate also other transcription factors. The functional diversity of coregulators reflects the many ways in which coregulators can enhance or repress AR-dependent transcription, which range from facilitating access to chromatin, ensuring proper AR conformation, modulating AR turnover, etc. The ability of these proteins to selectively control the level of androgen regulation of different AR target genes has been recognized for a while but is yet to be fully appreciated. Until recently, the unavailability of genome-wide AR and coregulator-dependent transcriptomes and cistromes has limited most evaluations to a few ARE-driven promoter-reporter genes and a handful of well-characterized AR target genes (e.g. Marshall et al. 2003, Heemers et al. 2009, Ianculescu et al. 2012). Genome-wide expression profiling and ChIP-based mapping of transcriptional regulators have now become

Table 1 Representative coregulators that are overexpressed in CR-CaP.

Coregulator	Key reference
BAF57 (<i>SMARCE1</i>)	Balasubramaniam et al. (2013)
CBP (<i>CREBBP</i>)	Comuzzi et al. (2004)
DNA-PK (<i>PRKDC</i>)	Goodwin et al. (2014)
EZH2	Varambally et al. (2002)
MAGEA11	Karpf et al. (2009)
MEP50 (<i>WDR77</i>)	Peng et al. (2008)
MLL2 (<i>KMT2</i>)	Malik et al. (2015)
PIAS1	Puhr et al. (2014)
PIRH2 (<i>RCHY1</i>)	Logan et al. (2006)
SRC1 (<i>NCOA1</i>)	Gregory et al. (2001)
TIF2 (<i>NCOA2</i>)	Agoulnik et al. (2006)
TIP60 (<i>KAT5</i>)	Halkidou et al. (2003)
TRIM24	Groner et al. (2016)
USP22	Schrecengost et al. (2014)

In italics, official gene symbol for coregulators for which the referenced literature report does not use the official gene symbol but an alias. Key reference, representative literature report that describes immunohistochemical overexpression of the coregulator in clinical CR-CaP specimens.

standard assays; these techniques permit better capture of the intricacies of coregulators' context-dependent and gene-specific regulation of AR transactivation. A few examples of different mechanisms by which select interaction between AR and its coregulators controls androgen-responsiveness of subsets of AR target genes are discussed in this section. Cases that are selected demonstrate for instance the ability of AR-associated coregulators to act simultaneously as a coactivator for some AR target genes and as a corepressor for others. Other examples document a change in function of the same coregulator from repressor to activator of gene transcription in clinical CaP before and after ADT, or point toward specific coregulator-AR interaction hubs at select AREs that control expression of only a subset of target genes.

LSD1 Lysine specific demethylase 1, or LSD1, can act as a coactivator or a corepressor for different sets of AR target genes, and both these regulatory functions are relevant to CaP progression (Metzger et al. 2010, Cai et al. 2011, 2014). The relevance of its corepressor function was highlighted following the identification of a novel ARE in the second exon of the AR gene (Cai et al. 2011). This ARE, which is distinct from the ARE that had been identified before in the AR gene 5'UTR (Wang et al. 2008b), is highly conserved among humans, mice and rats, and mediated active transcriptional repression of AR. Strikingly, this repression occurred at higher doses of androgens than those needed to induce expression of other AR target genes such as PSA. DHT treatment induced tight AR binding to this site, to which FoxA1, Oct1 and GATA2 were also recruited. Stimulation of cells with DHT caused a decrease in H3K4me1 and H3K4me2 epigenetic marks specifically at this AR binding site, but not at other AREs in the AR gene or at other representative AR target genes. The histone demethylase LSD1 was found to be recruited to this site after androgen stimulation and association of both AR and LSD1 with this site decreased after androgen deprivation of CaP cell lines and xenografts. Loss of LSD1 did not decrease DHT-stimulated recruitment of AR but did prevent the decrease in methylation at this site. LSD1's corepressor function for androgen regulation of the AR gene was extrapolated to other genes such as the gene encoding AKR1C3, a critical androgen biosynthesis enzyme for which expression increases under ADT in clinical CaP (Adeniji et al. 2013). These data indicated that lower androgen levels such as those found in CR-CaP are adequate to stimulate AR action on enhancer elements of genes mediated important metabolic functions, but are

not sufficient to reduce AR gene expression and cellular proliferation (Cai *et al.* 2011). These data also implied that the association between AR and LSD1, which had been reported as coactivator for other AR target genes (Metzger *et al.* 2005, 2010), does not determine LSD1's action as coactivator or corepressor. That distinction may be determined by properties of the DNA segment to which LSD1 binds. This hypothesis was supported by the previous literature reports in which LSD1 served as coactivator for AR target genes such as PSA and KLK2. LSD1's switch from an AR corepressor to an activator has been linked to its differential demethylase activity; i.e. removing mono- and dimethyl marks from H3K4 as a corepressor to removing mono- and dimethyl marks from H3K9 as coactivator, respectively. Phosphorylation of H3T6 by protein kinase C beta I has been identified as the key event that prevents LSD1 from demethylating H3K4 during AR-dependent gene activation (Metzger *et al.* 2010). Consistent with this model, ChIP-Seq studies showed that 20% of genome-wide LSD1 and AR peaks overlapped in androgen-treated LNCaP cells and co-occupied sites showed significant enrichment for H3T6ph marks (Cai *et al.* 2014). These observations provide an explanation for the distinct actions of LSD1 at subclasses of differentially epigenetically marked AREs to either repress or activate target gene transcription.

EZH2 Enhancer of zeste homolog 2, or EZH2, is of considerable interest in CaP as it is overexpressed in patient specimens, particularly in CR-CaP samples, where cells rely on it to proliferate (Varambally *et al.* 2002, Xu *et al.* 2012). As the catalytic subunit for polycomb repressive complex 2 (PRC2), EZH2 has been studied intensively for its role in gene repression, e.g. that of the tumor suppressor DAB2IP, via trimethylation of H3K27 (Cao *et al.* 2002, Chen *et al.* 2005). A comparison of EZH2 function between LNCaP cells and its CR subline abl, uncovered a novel, CR-CaP-specific role as an AR-associated coactivator (Xu *et al.* 2012). EZH2-dependent transcriptome profiling identified a larger gene set of EZH2-stimulated genes in abl and other CR-CaP cells compared to ADT-naïve cells, and these genes were highly expressed also in clinical CR-CaP clinical specimens. Subsequent ChIP-Seq analysis isolated subsets of EZH2 binding sites that lack H3K27 methylation marks, again selectively in CR-CaP cells. These peaks were enriched in epigenetic markers of active transcription, and the associated genes were induced by EZH2 and had prognostic power in CR-CaP. A striking enrichment for AR binding motifs was found in the CR-CaP-specific EZH2 binding sites. The physical interaction between AR and

EZH2, found in co-immunoprecipitation and gel filtration assays, was reflected in significant overlap in AR and EZH2 genomic binding peaks. Silencing of EZH2 selectively prevented binding of AR to co-occupied binding sites but not to other peaks. This shift in function of EZH2 from a more general transcriptional repressor to an AR-associated coactivator was caused by a phosphorylation of EZH2 at Ser21, which occurred specifically in CR-CaP cells and may be mediated by Akt action (Xu *et al.* 2012). This CaP stage-selective role for EZH2 implies that targeting this specific aspect of its function, which is not pursued by available EZH2 inhibitors (Sparmann & van Lohuizen 2006), may lead to a novel CR-specific CaP treatment modality.

SIAH2 Siah2 is an E3 ubiquitin ligase that targets a select pool of chromatin-bound AR for degradation, which leads to preferential control over the growth, survival and tumorigenic capacity of CaP cells, particularly under ADT conditions (Qi *et al.* 2013). Initial studies in which Siah2^{-/-} mice were crossed with TRAMP mice showed a greater reduction in expression of AR target genes such as NKX3.1 and SPINK3 but not other genes like probasin and TMPRSS2 in castrated Siah2-deficient animals. The suggestion that Siah2 regulates the expression of a specific subset of AR target genes was confirmed in human LNCaP and Rv1 CaP cell lines. Gene expression profiling on Rv1 cells after silencing of Siah2 expression showed downregulation of AR target genes with functions in lipid metabolism, steroid metabolism and cholesterol metabolism. Several of these genes were necessary for growth under ADT and were enriched in CR-CaP tissues. Siah2 bound to AR, and degraded AR in an E3 ubiquitin ligase-dependent manner; this Siah2-AR interaction was necessary for AR's transcriptional activity and control over Siah2-dependent AR target genes. siRNA-mediated silencing of Siah2 did not affect the levels of total nuclear- or chromatin-bound AR in CaP cells. It did, however, increase the levels of AR and NCoR1, a AR-associated corepressor and substrate for Siah2, at AREs of the select genes, but did not affect their binding to others, suggesting that Siah2 selectively degrades NCoR1-bound AR at those target genes. In line with this model, levels of ubiquitinated AR were reduced at those AREs after silencing of Siah2, and knock-down of NCoR1 attenuated Siah2 recruitment to those sites. Co-IP showed stronger association of Siah2 with NCoR1-bound AR than with AR alone (Qi *et al.* 2013). Siah2 thus uses another molecular route to diversify AR's transcriptional output, namely degradation of transcriptionally inactive, NCoR1-bound AR on AREs of select AR target genes. The 3

examples of coregulators that are discussed in this section provide a snapshot only of the variety in molecular mechanisms by which coregulators can influence the expression of subsets AR target genes.

Different molecular modes of AR action: opportunities for novel AR-targeting therapies?

Taken together, the evidence discussed above indicates that variability in the sequence of the ARE half-site(s) and the composition of the surrounding DNA regions at different target genes may dictate, or restrict, access of AR to DNA. In addition, differential contribution of pioneer factors has been linked to (re)programming of the AR cistrome. Non-pioneering transcription factors and coregulators use a variety of molecular mechanisms to modulate the output of AR transactivation at a given target gene and, thus induce or repress specific biological processes. These data provide evidence for the diverse composition, and subsequent output, of AR transcriptional complexes across AREs. This possibility fits with the previously proposed model of allosteric effects of DNA sequence on the 3-dimensional structure of DNA-bound GR, a close relative of AR. These allosteric effects have been linked with selective interaction between GR and the coregulators Brm and CARM1 (Meijsing et al. 2009). The observed specificity recalls also previously described allosteric effects in AR following binding by different ligands, which led to preferential interaction with different coregulators and coregulator-derived peptides (Baek et al. 2006, Norris et al. 2009b). The literature that is reviewed here suggests that allosteric effects with consequences for AR's interaction with transactivation factors may occur also when AR that has been stimulated by the same ligand is bound to different AREs.

The examples discussed here cover only a handful of known AR-affiliated transcription factors and coregulators. As genomic data for additional transcriptional regulators becomes available, it will be important to determine whether similar AR target gene specificity and context-dependency of AR's transcriptional output also occurs, to define the extent of overlap of such specificity for different AR-associated proteins, and to decipher these patterns in clinical specimens as well as preclinical models. The resulting datasets should increase significantly our knowledge about the modes of protein-protein interaction, and modular hierarchy that may govern such interactions. Modular interaction has been described

for steady-state coregulator complexes in HeLa cells (Malovannaya et al. 2011), but is yet to be examined with respect to ligand-activated transcription.

None-the-less, the evidence derived from the examples discussed here and from others that are not highlighted in this review (e.g. Xu et al. 2009, Toropainen et al. 2015), does support the presence of different hubs of AR-dependent transcription that control different aspects of androgen-dependent CaP cell biology. The existence of multiple transcriptional codes that result from specific AR-protein and AR-DNA interactions provides the possibility of targets other than the ligand-activation domain of AR to inhibit specific portion(s) of AR action that drives CaP progression (Heemers 2014). The opportunity to design those treatment modalities to only the select AR-dependent events that are CaP-specific may also reduce the severe side effects that are associated with systemic inhibition of AR action (Nguyen et al. 2014).

How to go about the daunting task of disrupting the output from specific AR transcriptional complexes? Some drug options may be available already. For instance, because a significant portion of them contains enzymatic moieties, coregulators have long been considered as attractive novel targets for CaP treatment (Chmelar et al. 2007, Heemers & Tindall 2010). For several coregulators, such as SRC family members and LSD1, inhibitors are available that have been tested in preclinical CaP models (Cai et al. 2011, 2014, Wang et al. 2011b, 2014). Other therapeutics may be derived from Chem-Seq approaches that map genome-wide interactions between small molecules and their protein targets on chromatin. Chem-Seq assays have identified e.g. SD70 as a novel inhibition of KDM4C to inhibit AR function and CaP cell growth (Jin et al. 2014). In the case of EZH2, novel inhibitors that target only its Ser21 phosphorylated activator function may lead to CaP stage-specific application (Xu et al. 2012). A potential drawback of this type of compounds is that they will likely simultaneously inhibit both coactivator and corepressor function of the coregulator that is being targeted. This may not be desirable, and/or may impact also on the action of other transcription factors with which the coregulator collaborates. The latter 'promiscuity' may lead to significant off-target effects. Other approaches to target more specifically discrete coregulatory-AR interactions of interest have been tested. The use of peptides, such as BAF57 inhibitory peptide (BIPep), which targets the protein-protein interfaces between AR and BAF57, blocks AR residency on chromatin, the resultant AR target gene activation and CaP cell proliferation (Link et al.

2005). Along the same lines, peptidomimetics have been developed that are able to disrupt interaction between AR and its coregulator PELP1 in several preclinical CaP models and *ex vivo* CaP explants (Ravindranathan *et al.* 2013). Similarly, multivalent peptoid conjugates that block AR coactivator–peptide interactions and prevent AR intermolecular interactions reduced proliferation of CR-CaP cell lines and reduced tumor growth, and AR and Ki67 expression in CR-CaP xenografts (Levine *et al.* 2012, Wang *et al.* 2016). While the above-mentioned tactics strive to disrupt specific AR–protein interactions, successful attempts have been made also at disruption the interaction between AR and AREs and between AR-associated transcription factors and their consensus binding motifs. DNA-binding polyamides that target the consensus ARE half-sites in genes such as PSA, reduce AR occupancy at AREs and effectively block the resulting gene transcription and exert antitumor effects on CaP xenografts (Nickols & Dervan 2007, Hargrove *et al.* 2015). Similar approaches in which the Oct1 binding site of a representative AR target gene was targeted also prevented Oct1-dependent transcription as well as the growth of CaP xenografts (Obinata *et al.* 2016). Alternatively, a small molecule that selectively binds a surface exposed pocket on AR DBD effectively blocked transcriptional activity of full-length AR and constitutively active AR splice variants (Dalal *et al.* 2014). Targeting what were previously viewed as undruggable interactions within transcriptional complexes provides proof-of-principle for development of novel compounds that inhibit critical protein–protein and protein–DNA interactions that are most relevant to AR-mediated lethal CaP progression. It is conceivable that similar approaches could be extrapolated to disrupt other relevant AR-coregulator, AR-transcription factor, coregulator-coregulator, etc. interactions (Fig. 4). Whether such compounds are best administered as monotherapy or as part of a more ‘holistic’ approach in which multiple interaction are targeted at the same time will need to be determined.

The primary goal of disruption crucial protein–protein and/or protein–DNA interactions is to impair the activity of the corresponding AR transcriptional complexes and to decrease their transcriptional output. Because such interventions may have secondary effects on the stability or the recruitment of different components of the AR complex at the set of AREs that is targeted, it will be important to monitor also their impact on AR, coregulator, transcription factor, RNA pol II etc., cistromes.

Confounders and facilitators of ‘selective’ forms of androgen deprivation therapy

From a molecular perspective, the output of a specific AR transcriptional complex can be influenced by alterations in the expression of AR, the DNA sequence to which it binds and its protein interactome. In clinical CaP, all of these components have been reported to undergo somatic alterations in a subset of cases. For instance, overexpression of AR and/or (co-) expression of AR variants that lack ligand binding domain impact the level of AR binding throughout the genome and composition of the AR cistrome (Urbanucci *et al.* 2012a,b, Chan *et al.* 2015, Lu *et al.* 2015). Copy number alterations occur in ARE-containing DNA regions and single nucleotide variants affect AREs (Whittington *et al.* 2016); both can impact the androgen-dependent expression of the corresponding ARE-containing target gene. Some AR target genes harbor more than 1 ARE, and long distance interactions between AR-bound enhancers with gene promoters mediate transcription of AR target genes (Reid *et al.* 2001, Wang *et al.* 2005). How the composition of the AR transcriptional complex correlates at different regions of the same gene, and the impact on its activity on cooperativity between multiple AREs within the same gene remains to be determined.

Defining the DNA binding patterns of components of AR transcriptional complexes, particularly those that do not directly bind DNA, in clinical CaP specimens has

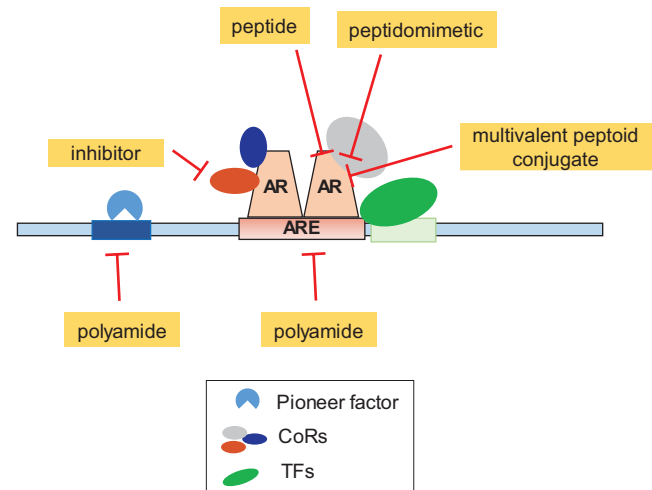


Figure 4

Overview of novel therapeutic strategies to disrupt select AR transcriptional complexes. Polyamides inhibit the binding of transcription factors with chromatin. Specific inhibitors target enzymatic moieties of some coregulators. Peptides, peptidomimetics and multivalent peptoid conjugates disrupt specific AR-coregulator interactions.

been challenging technically because of the amounts and the purity of tissue that is required. Nonetheless, efforts to define the AR cistrome in clinical CaP specimens have yielded novel insights in AR action in clinical CaP progression (Sharma *et al.* 2013, Pomerantz *et al.* 2015). Elucidation of the recruitment to DNA by AR-associated proteins is likely to be facilitated by the increasing availability of *ex vivo* explants, organoids, and patient-derived xenografts derived from localized and metastatic CaP that are representative of clinical CaP tissue. Unlike patient specimens, the latter models have the advantage that they allow for androgen supplementation or deprivation, or for silencing or overexpression of genes of interest.

Several AR-interacting proteins undergo somatic mutations that affect their regulatory function during progression to CR-CaP, including MLL2, NCOR1 and FoxA1 (Barbieri *et al.* 2012, Grasso *et al.* 2012). The impact of these alterations on AR-dependent target gene expression and AR cistrome has yet to be fully explored. It is likely, however, that these may have pronounced effects on AR's function and underlie the heterogeneity that has been observed for AR transcriptional output in patient specimens. As for genomic CaP heterogeneity in general, this variability is more pronounced in localized ADT-naïve CaP than in metastatic CaP that has recurred under ADT (Kumar *et al.* 2016, Wei *et al.* 2016). In addition to alterations that directly affect components of, and contributors to, AR-transcription complexes, the expression and mutational status of other genomic markers can affect AR's transactivation function. For instance, recurrent point mutations in the E3 ubiquitin ligase SPOP, found in up to 15 percent of CaP specimens (Barbieri *et al.* 2012), render SPOP incapable of degrading AR, AR variants and AR coregulators such as SRC3 and TRIM24 (Barbieri *et al.* 2012, Geng *et al.* 2013, An *et al.* 2014, Cancer Genome Atlas Research Network. Electronic address scmo & Cancer Genome Atlas Research Network 2015, Groner *et al.* 2016). As a consequence, expression of these proteins is elevated in CaPs that express mutant SPOP, which likely impacts the specific AR target gene expression profiles under their control. Alternatively, the TMPRSS2-ERG gene fusion is present in at least half of clinical CaPs (Bostrom *et al.* 2015). As discussed above, ERG contributes to transcriptional output of a subset of AR target genes in addition to repressing AR expression (Yu *et al.* 2010). When PTEN is deleted, the impact of TMPRSS2-ERG on aggressive CaP progression is even more pronounced (Zong *et al.* 2009, Chen *et al.* 2013); it is

possible that alterations in AR functions contribute to this behavior. The tumor suppressor Rb negatively regulates AR expression. Consequently, deletion of Rb, a common occurrence in CaP progression, increases significantly AR's expression and output (Sharma *et al.* 2010).

In view of the large number of AR-associated proteins, it is very likely that more than one 'selective' AR-dependent transcriptional mechanism is active in a given CaP. Deciding which mechanism(s) to pursue for therapeutic intervention may, therefore, not be straightforward. Therapeutic benefit will be most pronounced if the most appropriate mechanism(s) is targeted in a rational, evidence-based manner. The presence of the genomic alterations described above may serve as biomarkers to facilitate the decision to target, or avoid targeting, specific protein-protein or protein-DNA interactions. These genomic markers are measured in routine genomic tests that are performed on clinical CaP biopsies or radical prostatectomy specimens, such as the FoundationOne assay (Frampton *et al.* 2013). Alternatively, information on the expression level and post-translational modification or mutational status of AR-associated proteins, as well as the specific gene expression programs under their control, may also serve as suitable biomarkers. Moving forward, other potential contributors to AR-mediated transcriptional complexes such as lncRNAs, miRNAs, and pseudogenes, which could impact on the expression levels of the resulting AR mRNA profiles (Tay *et al.* 2014), may have to be considered also for the proposed therapeutic strategies. At this time, the knowledge on their respective contribution to specific AR-dependent transcriptional events is too limited for inclusion in the current review.

The proposed therapeutic strategies to target specific AR-dependent transcriptional complexes will require also appropriate clinical endpoints to monitor a patient's response and CaP progression. Serum levels of PSA, an AR target gene whose expression is evaluated as surrogate for CaP burden during CaP progression, may not be the most suitable measurement. Serum PSA has been recognized to be an imperfect CaP biomarker (Prensner *et al.* 2012). More relevant to this discussion, its expression may not be controlled to the same extent by different AR transcriptional complexes. Determination of the efficacy of the proposed therapies may benefit from disease monitoring as outlined by the Prostate Cancer Clinical Trials Working Group 3 (PCWG3) (Scher *et al.* 2016). PCWG3 has taken into consideration evolving CaP treatments and changes in drug development to formulate recommendations that facilitate disease

management, clinical decisions and clinical trial design. In addition to biochemical endpoints such as serum levels of PSA, PCWG3 guidelines include evaluation of the number of circulating tumor cells, (radiographic) imaging, and symptomatic progression, as well as taking into account the time to changes in these parameters. Assessment of the response to targeted therapies will benefit also from PCWG3-recommended serial molecular profiling of blood, circulating tumor cells or metastatic tumor sites. Measuring expression of specific target genes or gene signatures in blood, circulating tumor cells or CR-CaP tissue is expected to facilitate the development of predictive biomarkers of response to the proposed therapeutic avenues.

Conclusions

A growing body of evidence indicates that AR-dependent transcription does not occur via a uniform mechanism. Considerable variability in AR–DNA and AR–protein interactions exists that fine-tunes the output of specific AR's transcriptional complexes. Specific disruption of those interactions that control aggressive AR-dependent CaP progression may lead to a viable therapeutic strategy in CaP for which ADT that targets androgen biosynthesis has failed.

Declaration of interest

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

Funding

This work was supported by the National Cancer Institute (grant number CA166440), the Department of Defense Prostate Cancer Research Program (grant number W81XWH-16-1-0404), and VeloSano 3 pilot Research Award (to H V H).

Acknowledgements

The authors thank the members of the Heemers Laboratory for helpful discussions.

References

Adeniji AO, Chen M & Penning TM 2013 AKR1C3 as a target in castrate resistant prostate cancer. *Journal of Steroid Biochemistry and Molecular Biology* **137** 136–149. (doi:10.1016/j.jsbmb.2013.05.012)

Agoulnik IU, Vaid A, Nakka M, Alvarado M, Bingman WE 3rd, Erdem H, Frollov A, Smith CL, Ayala GE, Ittmann MM, et al. 2006 Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. *Cancer Research* **66** 10594–10602. (doi:10.1158/0008-5472.CAN-06-1023)

An J, Wang C, Deng Y, Yu L & Huang H 2014 Destruction of full-length androgen receptor by wild-type SPOP, but not prostate-cancer-associated mutants. *Cell Reports* **6** 657–669. (doi:10.1016/j.celrep.2014.01.013)

Andersen RJ, Mawji NR, Wang J, Wang G, Haile S, Myung JK, Watt K, Tam T, Yang YC, Banuelos CA, et al. 2010 Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. *Cancer Cell* **17** 535–546. (doi:10.1016/j.ccr.2010.04.027)

Attard G, Cooper CS & de Bono JS 2009 Steroid hormone receptors in prostate cancer: a hard habit to break? *Cancer Cell* **16** 458–462. (doi:10.1016/j.ccr.2009.11.006)

Attard G, Parker C, Eeles RA, Schroder F, Tomlins SA, Tannock I, Drake CG & de Bono JS 2016 Prostate cancer. *Lancet* **387** 70–82. (doi:10.1016/S0140-6736(14)61947-4)

Baek SH, Ohgi KA, Nelson CA, Welsbie D, Chen C, Sawyers CL, Rose DW & Rosenfeld MG 2006 Ligand-specific allosteric regulation of coactivator functions of androgen receptor in prostate cancer cells. *PNAS* **103** 3100–3105. (doi:10.1073/pnas.0510842103)

Baena E, Shao Z, Linn DE, Glass K, Hamblen MJ, Fujiwara Y, Kim J, Nguyen M, Zhang X, Godinho FJ, et al. 2013 ETV1 directs androgen metabolism and confers aggressive prostate cancer in targeted mice and patients. *Genes and Development* **27** 683–698. (doi:10.1101/gad.211011.112)

Balasubramaniam S, Comstock CE, Ertel A, Jeong KW, Stallcup MR, Addya S, McCue PA, Ostrander WF Jr, Augello MA & Knudsen KE 2013 Aberrant BAF57 signaling facilitates prometastatic phenotypes. *Clinical Cancer Research* **19** 2657–2667. (doi:10.1158/1078-0432.CCR-12-3049)

Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, White TA, Stojanov P, Van Allen E, Stransky N, et al. 2012 Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nature Genetics* **44** 685–689. (doi:10.1038/ng.2279)

Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, MacDonald TY, Jarosz M, Lipson D, Tagawa ST, Nanus DM, et al. 2013 Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. *European Urology* **63** 920–926. (doi:10.1016/j.eururo.2012.08.053)

Bostrom PJ, Bjartell AS, Catto JW, Eggener SE, Lilja H, Loeb S, Schalken J, Schlomm T & Cooperberg MR 2015 Genomic predictors of outcome in prostate cancer. *European Urology* **68** 1033–1044. (doi:10.1016/j.eururo.2015.04.008)

Boutros PC, Fraser M, Harding NJ, de Borja R, Trudel D, Lalonde E, Meng A, Hennings-Yeomans PH, McPherson A, Sabelnykova VY, et al. 2015 Spatial genomic heterogeneity within localized, multifocal prostate cancer. *Nature Genetics* **47** 736–745. (doi:10.1038/ng.3315)

Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, Nelson PS, Liu XS, Brown M & Balk SP 2011 Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. *Cancer Cell* **20** 457–471. (doi:10.1016/j.ccr.2011.09.001)

Cai C, He HH, Gao S, Chen S, Yu Z, Gao Y, Chen S, Chen MW, Zhang J, Ahmed M, et al. 2014 Lysine-specific demethylase 1 has dual functions as a major regulator of androgen receptor transcriptional activity. *Cell Reports* **9** 1618–1627. (doi:10.1016/j.celrep.2014.11.008)

Callewaert L, Verrijdt G, Christiaens V, Haelens A & Claessens F 2003 Dual function of an amino-terminal amphipathic helix in androgen receptor-mediated transactivation through specific and nonspecific response elements. *Journal of Biological Chemistry* **278** 8212–8218. (doi:10.1074/jbc.M210744200)

Cancer Genome Atlas Research Network. Electronic address scmo & Cancer Genome Atlas Research Network 2015 The molecular taxonomy of primary prostate cancer. *Cell* **163** 1011–1025.

Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS & Zhang Y 2002 Role of histone H3 lysine 27 methylation in

- Polycomb-group silencing. *Science* **298** 1039–1043. (doi:10.1126/science.1076997)
- Cao B, Qi Y, Zhang G, Xu D, Zhan Y, Alvarez X, Guo Z, Fu X, Plymate SR, Sartor O, et al. 2014 Androgen receptor splice variants activating the full-length receptor in mediating resistance to androgen-directed therapy. *Oncotarget* **5** 1646–1656. (doi:10.18632/oncotarget.1802)
- Celis L, Claessens F, Peeters B, Heyns W, Verhoeven G & Rombauts W 1993 Proteins interacting with an androgen-responsive unit in the C3(1) gene intron. *Molecular and Cellular Endocrinology* **94** 165–172. (doi:10.1016/0303-7207(93)90165-G)
- Chan SC, Selth LA, Li Y, Nyquist MD, Miao L, Bradner JE, Raj GV, Tilley WD & Dehm SM 2015 Targeting chromatin binding regulation of constitutively active AR variants to overcome prostate cancer resistance to endocrine-based therapies. *Nucleic Acids Research* **43** 5880–5897. (doi:10.1093/nar/gkv262)
- Chen H, Tu SW & Hsieh JT 2005 Down-regulation of human DAB2IP gene expression mediated by polycomb Ezh2 complex and histone deacetylase in prostate cancer. *Journal of Biological Chemistry* **280** 22437–22444. (doi:10.1074/jbc.M501379200)
- Chen Y, Chi P, Rockowitz S, Iaquina PJ, Shamu T, Shukla S, Gao D, Sirota I, Carver BS, Wongvipat J, et al. 2013 ETS factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to PTEN loss. *Nature Medicine* **19** 1023–1029. (doi:10.1038/nm.3216)
- Chen Z, Lan X, Thomas-Ahner JM, Wu D, Liu X, Ye Z, Wang L, Sunkel B, Grenade C, Chen J, et al. 2015 Agonist and antagonist switch DNA motifs recognized by human androgen receptor in prostate cancer. *EMBO Journal* **34** 502–516. (doi:10.15252/embj.201490306)
- Chmelar R, Buchanan G, Need EF, Tilley W & Greenberg NM 2007 Androgen receptor coregulators and their involvement in the development and progression of prostate cancer. *International Journal of Cancer* **120** 719–733. (doi:10.1002/ijc.22365)
- Claessens F, Joniau S & Helsen C 2017 Comparing the rules of engagement of androgen and glucocorticoid receptors. *Cellular and Molecular Life Sciences* **74** 2217–2228. (doi:10.1007/s00018-017-2467-3)
- Comuzzi B, Nemes C, Schmidt S, Jasarevic Z, Lodde M, Pycha A, Bartsch G, Offner F, Culig Z & Hobisch A 2004 The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. *Journal of Pathology* **204** 159–166. (doi:10.1002/path.1609)
- Culig Z 2004 Androgen receptor cross-talk with cell signalling pathways. *Growth Factors* **22** 179–184. (doi:10.1080/08977190412331279908)
- Dai C, Heemers HV & Sharifi N 2017 Androgen signalling in prostate cancer. *Cold Spring Harbor Perspectives in Medicine* pii: a030452. (doi:10.1101/cshperspect.a030452)
- Dalal K, Roshan-Moniri M, Sharma A, Li H, Ban F, Hassona MD, Hsing M, Singh K, LeBlanc E, Dehm S, et al. 2014 Selectively targeting the DNA-binding domain of the androgen receptor as a prospective therapy for prostate cancer. *Journal of Biological Chemistry* **289** 26417–26429. (doi:10.1074/jbc.M114.553818)
- De Laere B, van Dam PJ, Whittington T, Mayrhofer M, Diaz EH, Van den Eynden G, Vandebroek J, Del-Favero J, Van Laere S, Dirix L, et al. 2017 Comprehensive profiling of the androgen receptor in liquid biopsies from castration-resistant prostate cancer reveals novel intra-AR structural variation and splice variant expression patterns. *European Urology* pii S0302-2838(17)30018-0. (doi:10.1016/j.eururo.2017.01.011)
- Dehm SM, Schmidt LJ, Heemers HV, Vessella RL & Tindall DJ 2008 Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Research* **68** 5469–5477. (doi:10.1158/0008-5472.CAN-08-0594)
- Denmeade SR & Isaacs JT 2002 A history of prostate cancer treatment. *Nature Reviews Cancer* **2** 389–396. (doi:10.1038/nrc801)
- DePriest AD, Fiandalo MV, Schlanger S, Heemers F, Mohler JL, Liu S & Heemers HV 2016 Regulators of androgen action resource: a one-stop shop for the comprehensive study of androgen receptor action. *Database* 2016.
- Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, Schnall-Levin M, White J, Sanford EM, An P, et al. 2013 Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nature Biotechnology* **31** 1023–1031. (doi:10.1038/nbt.2696)
- Geng C, He B, Xu L, Barbieri CE, Eedunuri VK, Chew SA, Zimmermann M, Bond R, Shou J, Li C, et al. 2013 Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. *PNAS* **110** 6997–7002. (doi:10.1073/pnas.1304502110)
- Goodwin JF, Schiewer MJ, Dean JL, Schrecengost RS, de Leeuw R, Han S, Ma T, Den RB, Dicker AP, Feng FY, et al. 2013 A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discovery* **3** 1254–1271. (doi:10.1158/2159-8290.CD-13-0108)
- Goodwin JF, Kothari V, Drake JM, Zhao S, Dylgjeri E, Dean JL, Schiewer MJ, McNair C, Jones JK, Aytes A, et al. 2015 DNA-PKcs-mediated transcriptional regulation drives prostate cancer progression and metastasis. *Cancer Cell* **28** 97–113. (doi:10.1016/j.ccell.2015.06.004)
- Gottlieb B, Beitel LK, Nadarajah A, Paliouras M & Trifiro M 2012 The androgen receptor gene mutations database: 2012 update. *Human Mutations* **33** 887–894. (doi:10.1002/humu.22046)
- Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, et al. 2012 The mutational landscape of lethal castration-resistant prostate cancer. *Nature* **487** 239–243. (doi:10.1038/nature11125)
- Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS & Wilson EM 2001 A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. *Cancer Research* **61** 4315–4319.
- Groner AC, Cato L, de Tribolet-Hardy J, Bernasocchi T, Janouskova H, Melchers D, Houtman R, Cato AC, Tschopp P, Gu L, et al. 2016 TRIM24 is an oncogenic transcriptional activator in prostate cancer. *Cancer Cell* **29** 846–858. (doi:10.1016/j.ccell.2016.04.012)
- Haelens A, Verrijdt G, Schoenmakers E, Alen P, Peeters B, Rombauts W & Claessens F 1999 The first exon of the human sc gene contains an androgen responsive unit and an interferon regulatory factor element. *Molecular and Cellular Endocrinology* **153** 91–102. (doi:10.1016/S0303-7207(99)00079-9)
- Halkidou K, Gnanapragasam VJ, Mehta PB, Logan IR, Brady ME, Cook S, Leung HY, Neal DE & Robson CN 2003 Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* **22** 2466–2477. (doi:10.1038/sj.onc.1206342)
- Hargrove AE, Martinez TF, Hare AA, Kurmis AA, Phillips JW, Sud S, Pienta KJ & Dervan PB 2015 Tumor repression of VCaP xenografts by a pyrrole-imidazole polyamide. *PLoS ONE* **10** e0143161. (doi:10.1371/journal.pone.0143161)
- He HH, Meyer CA, Shin H, Bailey ST, Wei G, Wang Q, Zhang Y, Xu K, Ni M, Lupien M, et al. 2010 Nucleosome dynamics define transcriptional enhancers. *Nature Genetics* **42** 343–347. (doi:10.1038/ng.545)
- He B, Lanz RB, Fiskus W, Geng C, Yi P, Hartig SM, Rajapakshe K, Shou J, Wei L, Shah SS, et al. 2014 GATA2 facilitates steroid receptor coactivator recruitment to the androgen receptor complex. *PNAS* **111** 18261–18266. (doi:10.1073/pnas.1421415111)
- Heemers HV 2014 Targeting androgen receptor action for prostate cancer treatment: does the post-receptor level provide novel opportunities? *International Journal of Biological Sciences* **10** 576–587. (doi:10.7150/ijbs.8479)
- Heemers H & Tindall D 2005 Androgen receptor coregulatory proteins as potential therapeutic targets in the treatment of prostate cancer. *Current Cancer Therapy Reviews* **1** 175–186. (doi:10.2174/1573394054021745)
- Heemers HV & Tindall DJ 2007 Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR

- transcriptional complex. *Endocrine Reviews* **28** 778–808. (doi:10.1210/er.2007-0019)
- Heemers H & Tindall D 2010 Nuclear receptor coregulators: promising therapeutic targets for the treatment of prostate cancer. In *Drug Management of Prostate Cancer*, pp 41–52. Eds WD Figg, CH Chau & E Small. New York, NY, USA: Springer.
- Heemers HV, Regan KM, Schmidt LJ, Anderson SK, Ballman KV & Tindall DJ 2009 Androgen modulation of coregulator expression in prostate cancer cells. *Molecular Endocrinology* **23** 572–583. (doi:10.1210/me.2008-0363)
- Heinlein CA & Chang C 2004 Androgen receptor in prostate cancer. *Endocrine Reviews* **25** 276–308. (doi:10.1210/er.2002-0032)
- Henzler C, Li Y, Yang R, McBride T, Ho Y, Sprenger C, Liu G, Coleman I, Lakely B, Li R, et al. 2016 Truncation and constitutive activation of the androgen receptor by diverse genomic rearrangements in prostate cancer. *Nature Communications* **7** 13668. (doi:10.1038/ncomms13668)
- Horie-Inoue K & Inoue S 2013 Genome-wide integrated analyses of androgen receptor signaling in prostate cancer based on high-throughput technology. *Current Drug Targets* **14** 472–480. (doi:10.2174/1389450111314040008)
- Hu R, Isaacs WB & Luo J 2011 A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *Prostate* **71** 1656–1667. (doi:10.1002/pros.21382)
- Huang L, Pu Y, Hepps D, Danielpour D & Prins GS 2007 Posterior Hox gene expression and differential androgen regulation in the developing and adult rat prostate lobes. *Endocrinology* **148** 1235–1245. (doi:10.1210/en.2006-1250)
- Huggins CS 1941 Studies on prostatic cancer: 2. the effects of castration on advanced carcinoma of the prostate gland. *Archives of Surgery* **43** 209–212. (doi:10.1001/archsurg.1941.01210140043004)
- Huggins C & Hodges CV 1941 Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Research* **1** 293–297.
- Ianculescu I, Wu DY, Siegmund KD & Stallcup MR 2012 Selective roles for cAMP response element-binding protein binding protein and p300 protein as coregulators for androgen-regulated gene expression in advanced prostate cancer cells. *Journal of Biological Chemistry* **287** 4000–4013. (doi:10.1074/jbc.M111.300194)
- Jeter CR, Badeaux M, Choy G, Chandra D, Patrawala L, Liu C, Calhoun-Davis T, Zaehres H, Daley GQ & Tang DG 2009 Functional evidence that the self-renewal gene NANOG regulates human tumor development. *Stem Cells* **27** 993–1005. (doi:10.1002/stem.29)
- Jeter CR, Liu B, Liu X, Chen X, Liu C, Calhoun-Davis T, Repass J, Zaehres H, Shen JJ & Tang DG 2011 NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation. *Oncogene* **30** 3833–3845. (doi:10.1038/onc.2011.114)
- Jeter CR, Liu B, Lu Y, Chao HP, Zhang D, Liu X, Chen X, Li Q, Rycaj K, Calhoun-Davis T, et al. 2016 NANOG reprograms prostate cancer cells to castration resistance via dynamically repressing and engaging the AR/FOXA1 signaling axis. *Cell Discovery* **2** 16041. (doi:10.1038/celldisc.2016.41)
- Jin HJ, Kim J & Yu J 2013 Androgen receptor genomic regulation. *Translational Andrology and Urology* **2** 157–177.
- Jin C, Yang L, Xie M, Lin C, Merkurjev D, Yang JC, Tanasa B, Oh S, Zhang J, Ohgi KA, et al. 2014 Chem-seq permits identification of genomic targets of drugs against androgen receptor regulation selected by functional phenotypic screens. *PNAS* **111** 9235–9240. (doi:10.1073/pnas.1404303111)
- Jozwik KM & Carroll JS 2012 Pioneer factors in hormone-dependent cancers. *Nature Review Cancer* **12** 381–385. (doi:10.1038/nrc3263)
- Jung C, Kim RS, Zhang HJ, Lee SJ & Jeng MH 2004 HOXB13 induces growth suppression of prostate cancer cells as a repressor of hormone-activated androgen receptor signaling. *Cancer Research* **64** 9185–9192. (doi:10.1158/0008-5472.CAN-04-1330)
- Karantanos T, Evans CP, Tombal B, Thompson TC, Montironi R & Isaacs WB 2015 Understanding the mechanisms of androgen deprivation resistance in prostate cancer at the molecular level. *European Urology* **67** 470–479. (doi:10.1016/j.eururo.2014.09.049)
- Karpf AR, Bai S, James SR, Mohler JL & Wilson EM 2009 Increased expression of androgen receptor coregulator MAGE-11 in prostate cancer by DNA hypomethylation and cyclic AMP. *Molecular Cancer Research* **7** 523–535. (doi:10.1158/1541-7786.MCR-08-0400)
- Klezovitch O, Risk M, Coleman I, Lucas JM, Null M, True LD, Nelson PS & Vasioukhin V 2008 A causal role for ERG in neoplastic transformation of prostate epithelium. *PNAS* **105** 2105–2110. (doi:10.1073/pnas.0711711105)
- Knudsen KE & Penning TM 2010 Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends in Endocrinology and Metabolism* **21** 315–324. (doi:10.1016/j.tem.2010.01.002)
- Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, Etzioni R, Bolouri H, Montgomery B, White T, et al. 2016 Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nature Medicine* **22** 369–378. (doi:10.1038/nm.4053)
- Levine PM, Imberg K, Garabedian MJ & Kirshenbaum K 2012 Multivalent peptidomimetic conjugates: a versatile platform for modulating androgen receptor activity. *Journal of the American Chemical Society* **134** 6912–6915. (doi:10.1021/ja300170n)
- Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, Liu J, Upadhyay SK, Auchus RJ & Sharifi N 2015 Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* **523** 347–351. (doi:10.1038/nature14406)
- Li Z, Alyamani M, Li J, Rogacki K, Abazeed M, Upadhyay SK, Balk SP, Taplin ME, Auchus RJ & Sharifi N 2016 Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature* **533** 547–551. (doi:10.1038/nature17954)
- Link KA, Burd CJ, Williams E, Marshall T, Rosson G, Henry E, Weissman B & Knudsen KE 2005 BAF57 governs androgen receptor action and androgen-dependent proliferation through SWI/SNF. *Molecular and Cellular Biology* **25** 2200–2215. (doi:10.1128/MCB.25.6.2200-2215.2005)
- Logan IR, Gaughan L, McCracken SR, Sapountzi V, Leung HY & Robson CN 2006 Human PIRH2 enhances androgen receptor signaling through inhibition of histone deacetylase 1 and is overexpressed in prostate cancer. *Molecular and Cellular Biology* **26** 6502–6510. (doi:10.1128/MCB.00147-06)
- Lu J, Van der Steen T & Tindall DJ 2015 Are androgen receptor variants a substitute for the full-length receptor? *Nature Reviews Urology* **12** 137–144. (doi:10.1038/nrurol.2015.13)
- Magnan S, Zarychanski R, Pilote L, Bernier L, Shemilt M, Vigneault E, Fradet V & Turgeon AF 2015 Intermittent vs continuous androgen deprivation therapy for prostate cancer: a systematic review and meta-analysis. *JAMA Oncology* **1** 1261–1269. (doi:10.1001/jamaoncol.2015.2895)
- Malik R, Khan AP, Asangani IA, Cieslik M, Prensner JR, Wang X, Iyer MK, Jiang X, Borkin D, Escara-Wilke J, et al. 2015 Targeting the MLL complex in castration-resistant prostate cancer. *Nature Medicine* **21** 344–352. (doi:10.1038/nm.3830)
- Malovannaya A, Lanz RB, Jung SY, Bulynko Y, Le NT, Chan DW, Ding C, Shi Y, Yucer N, Krenziute G, et al. 2011 Analysis of the human endogenous coregulator complexome. *Cell* **145** 787–799. (doi:10.1016/j.cell.2011.05.006)
- Marshall TW, Link KA, Petre-Draviam CE & Knudsen KE 2003 Differential requirement of SWI/SNF for androgen receptor activity. *Journal of Biological Chemistry* **278** 30605–30613. (doi:10.1074/jbc.M304582200)

- Massie CE, Adryan B, Barbosa-Morais NL, Lynch AG, Tran MG, Neal DE & Mills IG 2007 New androgen receptor genomic targets show an interaction with the ETS1 transcription factor. *EMBO Reports* **8** 871–878. (doi:10.1038/sj.embor.7401046)
- McNair C, Urbanucci A, Comstock CE, Augello MA, Goodwin JF, Launchbury R, Zhao SG, Schiewer MJ, Ertel A, Karnes J, et al. 2017 Cell cycle-coupled expansion of AR activity promotes cancer progression. *Oncogene* **36** 1655–1668. (doi:10.1038/onc.2016.334)
- Meijsing SH, Pufall MA, So AY, Bates DL, Chen L & Yamamoto KR 2009 DNA binding site sequence directs glucocorticoid receptor structure and activity. *Science* **324** 407–410. (doi:10.1126/science.1164265)
- Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH, Gunther T, Buettner R & Schule R 2005 LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **437** 436–439.
- Metzger E, Imhof A, Patel D, Kahl P, Hoffmeyer K, Friedrichs N, Muller JM, Greschik H, Kirfel J, Ji S, et al. 2010 Phosphorylation of histone H3T6 by PKCbeta(I) controls demethylation at histone H3K4. *Nature* **464** 792–796. (doi:10.1038/nature08839)
- Myung JK, Banuelos CA, Fernandez JG, Mawji NR, Wang J, Tien AH, Yang Y, Tavakoli I, Haile S, Watt K, et al. 2013 An androgen receptor N-terminal domain antagonist for treating prostate cancer. *Journal of Clinical Investigation* **123** 2948–2960. (doi:10.1172/JCI66398)
- Nguyen PL, Alibhai SM, Basaria S, D'Amico AV, Kantoff PW, Keating NL, Penson DF, Rosario DJ, Tombal B & Smith MR 2015 Adverse effects of androgen deprivation therapy and strategies to mitigate them. *European Urology* **67** 825–836. (doi:10.1016/j.eururo.2014.07.010)
- Nickols NG & Dervan PB 2007 Suppression of androgen receptor-mediated gene expression by a sequence-specific DNA-binding polyamide. *PNAS* **104** 10418–10423. (doi:10.1073/pnas.0704217104)
- Norris JD, Chang CY, Wittmann BM, Kunder RS, Cui H, Fan D, Joseph JD & McDonnell DP 2009a The homeodomain protein HOXB13 regulates the cellular response to androgens. *Molecular Cell* **36** 405–416. (doi:10.1016/j.molcel.2009.10.020)
- Norris JD, Joseph JD, Sherk AB, Juzumiene D, Turnbull PS, Rafferty SW, Cui H, Anderson E, Fan D, Dye DA, et al. 2009b Differential presentation of protein interaction surfaces on the androgen receptor defines the pharmacological actions of bound ligands. *Chemistry and Biology* **16** 452–460. (doi:10.1016/j.chembiol.2009.01.016)
- Obinata D, Takayama K, Fujiwara K, Suzuki T, Tsutsumi S, Fukuda N, Nagase H, Fujimura T, Urano T, Homma Y, et al. 2016 Targeting Oct1 genomic function inhibits androgen receptor signaling and castration-resistant prostate cancer growth. *Oncogene* **35** 6350–6358. (doi:10.1038/onc.2016.171)
- Peng Y, Chen F, Melamed J, Chiriboga L, Wei J, Kong X, McLeod M, Li Y, Li CX, Feng A, et al. 2008 Distinct nuclear and cytoplasmic functions of androgen receptor cofactor p44 and association with androgen-independent prostate cancer. *PNAS* **105** 5236–5241. (doi:10.1073/pnas.0712262105)
- Pomerantz MM, Li F, Takeda DY, Lenci R, Chonkar A, Chabot M, Cejas P, Vazquez F, Cook J, Shivdasani RA, et al. 2015 The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. *Nature Genetics* **47** 1346–1351. (doi:10.1038/ng.3419)
- Prensnor JR, Rubin MA, Wei JT & Chinnaiyan AM 2012 Beyond PSA: the next generation of prostate cancer biomarkers. *Science Translational Medicine* **4** 127rv123. (doi:10.1126/scitranslmed.3003180)
- Puhr M, Hofer J, Neuwirt H, Eder IE, Kern J, Schafer G, Geley S, Heidegger I, Klocker H & Culig Z 2014 PIAS1 is a crucial factor for prostate cancer cell survival and a valid target in docetaxel resistant cells. *Oncotarget* **5** 12043–12056. (doi:10.18632/oncotarget.2658)
- Qi J, Tripathi M, Mishra R, Sahgal N, Fazli L, Ettinger S, Placzek WJ, Claps G, Chung LW, Bowtell D, et al. 2013 The E3 ubiquitin ligase Siah2 contributes to castration-resistant prostate cancer by regulation of androgen receptor transcriptional activity. *Cancer Cell* **23** 332–346. (doi:10.1016/j.ccr.2013.02.016)
- Ravindranathan P, Lee TK, Yang L, Centenera MM, Butler L, Tilley WD, Hsieh JT, Ahn JM & Raj GV 2013 Peptidomimetic targeting of critical androgen receptor-coregulator interactions in prostate cancer. *Nature Communications* **4** 1923. (doi:10.1038/ncomms2912)
- Reid KJ, Hendy SC, Saito J, Sorensen P & Nelson CC 2001 Two classes of androgen receptor elements mediate cooperativity through allosteric interactions. *Journal of Biological Chemistry* **276** 2943–2952. (doi:10.1074/jbc.M009170200)
- Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, et al. 2015 Integrative clinical genomics of advanced prostate cancer. *Cell* **161** 1215–1228. (doi:10.1016/j.cell.2015.05.001)
- Sahu B, Laakso M, Ovaska K, Mirtti T, Lundin J, Rannikko A, Sankila A, Turunen JP, Lundin M, Konsti J, et al. 2011 Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. *EMBO Journal* **30** 3962–3976. (doi:10.1038/emboj.2011.328)
- Sahu B, Laakso M, Pihlajamaa P, Ovaska K, Sinielnikov I, Hautaniemi S & Janne OA 2013 FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. *Cancer Research* **73** 1570–1580. (doi:10.1158/0008-5472.CAN-12-2350)
- Sahu B, Pihlajamaa P, Dubois V, Kerkhofs S, Claessens F & Janne OA 2014 Androgen receptor uses relaxed response element stringency for selective chromatin binding and transcriptional regulation in vivo. *Nucleic Acids Research* **42** 4230–4240. (doi:10.1093/nar/gkt1401)
- Scarlett CO & Robins DM 1995 In vivo footprinting of an androgen-dependent enhancer reveals an accessory element integral to hormonal response. *Molecular Endocrinology* **9** 413–423. (doi:10.1210/mend.9.4.7659085)
- Scher HI, Morris MJ, Stadler WM, Higano C, Basch E, Fizazi K, Antonarakis ES, Beer TM, Carducci MA, Chi KN, et al. 2016 Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the prostate cancer clinical trials working group 3. *Journal of Clinical Oncology* **34** 1402–1418. (doi:10.1200/JCO.2015.64.2702)
- Schmidt LJ & Tindall DJ 2013 Androgen receptor: past, present and future. *Current Drug Targets* **14** 401–407. (doi:10.2174/1389450111314040002)
- Schreengost RS, Dean JL, Goodwin JF, Schiewer MJ, Urban MW, Stanek TJ, Sussman RT, Hicks JL, Birbe RC, Draganova-Tacheva RA, et al. 2014 USP22 regulates oncogenic signaling pathways to drive lethal cancer progression. *Cancer Research* **74** 272–286. (doi:10.1158/0008-5472.CAN-13-1954)
- Shaffer PL, Jivan A, Dollins DE, Claessens F & Gewirth DT 2004 Structural basis of androgen receptor binding to selective androgen response elements. *PNAS* **101** 4758–4763. (doi:10.1073/pnas.0401123101)
- Sharma A, Yeow WS, Ertel A, Coleman I, Clegg N, Thangavel C, Morrissey C, Zhang X, Comstock CE, Witkiewicz AK, et al. 2010 The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. *Journal of Clinical Investigation* **120** 4478–4492. (doi:10.1172/JCI44239)
- Sharma NL, Massie CE, Ramos-Montoya A, Zecchini V, Scott HE, Lamb AD, MacArthur S, Stark R, Warren AY, Mills IG, et al. 2013 The androgen receptor induces a distinct transcriptional program in castration-resistant prostate cancer in man. *Cancer Cell* **23** 35–47. (doi:10.1016/j.ccr.2012.11.010)
- Sharma NL, Massie CE, Butter F, Mann M, Bon H, Ramos-Montoya A, Menon S, Stark R, Lamb AD, Scott HE, et al. 2014 The ETS family member GABPalphamodulates androgen receptor signalling and mediates an aggressive phenotype in prostate cancer. *Nucleic Acids Research* **42** 6256–6269. (doi:10.1093/nar/gku281)

- Siegel RL, Miller KD & Jemal A 2017 Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians* **67** 7–30.
- Sparmann A & van Lohuizen M 2006 Polycomb silencers control cell fate, development and cancer. *Nature Reviews Cancer* **6** 846–856. (doi:10.1038/nrc1991)
- Steurer S, Mayer PS, Adam M, Krohn A, Koop C, Ospina-Klinck D, Tehrani AA, Simon R, Tennstedt P, Graefen M, et al. 2014 TMPRSS2-ERG fusions are strongly linked to young patient age in low-grade prostate cancer. *European Urology* **66** 978–981. (doi:10.1016/j.eururo.2014.06.027)
- Svensson MA, LaFargue CJ, MacDonald TY, Pflueger D, Kitabayashi N, Santa-Cruz AM, Garsha KE, Sathyanarayana UG, Riley JP, Yun CS, et al. 2011 Testing mutual exclusivity of ETS rearranged prostate cancer. *Laboratory Investigation* **91** 404–412. (doi:10.1038/labinvest.2010.179)
- Tadokoro-Cuccaro R, Davies J, Mongan NP, Bunch T, Brown RS, Audi L, Watt K, McEwan IJ & Hughes IA 2014 Promoter-dependent activity on androgen receptor N-terminal domain mutations in androgen insensitivity syndrome. *Sexual Development* **8** 339–349. (doi:10.1159/000369266)
- Tan PY, Chang CW, Chng KR, Wansa KD, Sung WK & Cheung E 2012 Integration of regulatory networks by NKX3-1 promotes androgen-dependent prostate cancer survival. *Molecular and Cellular Biology* **32** 399–414. (doi:10.1128/MCB.05958-11)
- Tay Y, Rinn J & Pandolfi PP 2014 The multilayered complexity of ceRNA crosstalk and competition. *Nature* **505** 344–352. (doi:10.1038/nature12986)
- Tewari AK, Yardimci GG, Shibata Y, Sheffield NC, Song L, Taylor BS, Georgiev SG, Coetzee GA, Ohler U, Furey TS, et al. 2012 Chromatin accessibility reveals insights into androgen receptor activation and transcriptional specificity. *Genome Biology* **13** R88. (doi:10.1186/gb-2012-13-10-r88)
- Thomas JD, Longen CG, Oyer HM, Chen N, Maher CM, Salvino JM, Kania B, Anderson KN, Ostrander WF, Knudsen KE, et al. 2017 Sigma1 targeting to suppress aberrant androgen receptor signaling in prostate cancer. *Cancer Research* **77** 2439–2452. (doi:10.1158/0008-5472.CAN-16-1055)
- Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, et al. 2005 Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310** 644–648. (doi:10.1126/science.1117679)
- Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, Cao Q, Prensner JR, Rubin MA, Shah RB, et al. 2008 Role of the TMPRSS2-ERG gene fusion in prostate cancer. *Neoplasia* **10** 177–188. (doi:10.1593/neo.07822)
- Toropainen S, Malinen M, Kaikkonen S, Rytinki M, Jaaskelainen T, Sahu B, Janne OA & Palvimo JJ 2015 SUMO ligase PIAS1 functions as a target gene selective androgen receptor coregulator on prostate cancer cell chromatin. *Nucleic Acids Research* **43** 848–861. (doi:10.1093/nar/gku1375)
- Urbanucci A, Marttila S, Janne OA & Visakorpi T 2012a Androgen receptor overexpression alters binding dynamics of the receptor to chromatin and chromatin structure. *Prostate* **72** 1223–1232. (doi:10.1002/pros.22473)
- Urbanucci A, Sahu B, Seppala J, Larjo A, Latonen LM, Waltering KK, Tammela TL, Vessella RL, Lahdesmaki H, Janne OA, et al. 2012b Overexpression of androgen receptor enhances the binding of the receptor to the chromatin in prostate cancer. *Oncogene* **31** 2153–2163. (doi:10.1038/onc.2011.401)
- Valenca LB, Sweeney CJ & Pomerantz MM 2015 Sequencing current therapies in the treatment of metastatic prostate cancer. *Cancer Treatment Reviews* **41** 332–340. (doi:10.1016/j.ctrv.2015.02.010)
- Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, et al. 2002 The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419** 624–629. (doi:10.1038/nature01075)
- Verrijdt G, Tanner T, Moehren U, Callewaert L, Haelens A & Claessens F 2006 The androgen receptor DNA-binding domain determines androgen selectivity of transcriptional response. *Biochemical Society Transactions* **34** 1089–1094. (doi:10.1042/BST0341089)
- Wang Q, Carroll JS & Brown M 2005 Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Molecular Cell* **19** 631–642. (doi:10.1016/j.molcel.2005.07.018)
- Wang Q, Li W, Liu XS, Carroll JS, Janne OA, Keeton EK, Chinnaiyan AM, Pienta KJ & Brown M 2007 A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. *Molecular Cell* **27** 380–392. (doi:10.1016/j.molcel.2007.05.041)
- Wang J, Cai Y, Yu W, Ren C, Spencer DM & Ittmann M 2008a Pleiotropic biological activities of alternatively spliced TMPRSS2/ERG fusion gene transcripts. *Cancer Research* **68** 8516–8524. (doi:10.1158/0008-5472.CAN-08-1147)
- Wang LG, Johnson EM, Kinoshita Y, Babb JS, Buckley MT, Liebes LF, Melamed J, Liu XM, Kurek R, Ossowski L, et al. 2008b Androgen receptor overexpression in prostate cancer linked to Pur alpha loss from a novel repressor complex. *Cancer Research* **68** 2678–2688. (doi:10.1158/0008-5472.CAN-07-6017)
- Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, Chen Z, Beroukhim R, Wang H, Lupien M, et al. 2009 Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. *Cell* **138** 245–256. (doi:10.1016/j.cell.2009.04.056)
- Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, et al. 2011a Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* **474** 390–394. (doi:10.1038/nature10006)
- Wang Y, Lonard DM, Yu Y, Chow DC, Palzkill TG & O'Malley BW 2011b Small molecule inhibition of the steroid receptor coactivators, SRC-3 and SRC-1. *Molecular Endocrinology* **25** 2041–2053. (doi:10.1210/me.2011-1222)
- Wang Y, Lonard DM, Yu Y, Chow DC, Palzkill TG, Wang J, Qi R, Matzuk AJ, Song X, Madoux F, et al. 2014 Bufalin is a potent small-molecule inhibitor of the steroid receptor coactivators SRC-3 and SRC-1. *Cancer Research* **74** 1506–1517. (doi:10.1158/0008-5472.CAN-13-2939)
- Wang Y, Dehigasptiya DC, Levine PM, Profit AA, Haugbro M, Imberg-Kazdan K, Logan SK, Kirshenbaum K & Garabedian MJ 2016 Multivalent peptoid conjugates which overcome enzalutamide resistance in prostate cancer cells. *Cancer Research* **76** 5124–5132. (doi:10.1158/0008-5472.CAN-16-0385)
- Wei L, Wang J, Lampert E, Schlanger S, DePriest AD, Hu Q, Gomez EC, Murakam M, Glenn ST, Conroy J, et al. 2017 Intratumoral and intertumoral genomic heterogeneity of multifocal localized prostate cancer impacts molecular classifications and genomic prognosticators. *European Urology* **71** 183–192. (doi:10.1016/j.eururo.2016.07.008)
- Weischenfeldt J, Simon R, Feuerbach L, Schlangen K, Weichenhan D, Minner S, Wuttig D, Warnatz HJ, Stehr H, Rausch T, et al. 2013 Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. *Cancer Cell* **23** 159–170. (doi:10.1016/j.ccr.2013.01.002)
- Whittington T, Gao P, Song W, Ross-Adams H, Lamb AD, Yang Y, Sveiza I, Klevebring D, Mills IG, Karlsson R, et al. 2016 Gene regulatory mechanisms underpinning prostate cancer susceptibility. *Nature Genetics* **48** 387–397. (doi:10.1038/ng.3523)
- Wilson S, Qi J & Filipp FV 2016 Refinement of the androgen response element based on ChIP-Seq in androgen-insensitive and androgen-responsive prostate cancer cell lines. *Scientific Reports* **6** 32611. (doi:10.1038/srep32611)
- Wu D, Sunkel B, Chen Z, Liu X, Ye Z, Li Q, Grenade C, Ke J, Zhang C, Chen H, et al. 2014 Three-tiered role of the pioneer factor GATA2 in promoting androgen-dependent gene expression in prostate

- cancer. *Nucleic Acids Research* **42** 3607–3622. (doi:10.1093/nar/gkt1382)
- Xu K, Shimelis H, Linn DE, Jiang R, Yang X, Sun F, Guo Z, Chen H, Li W, Kong X, et al. 2009 Regulation of androgen receptor transcriptional activity and specificity by RNF6-induced ubiquitination. *Cancer Cell* **15** 270–282. (doi:10.1016/j.ccr.2009.02.021)
- Xu K, Wu ZJ, Groner AC, He HH, Cai C, Lis RT, Wu X, Stack EC, Loda M, Liu T, et al. 2012 EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. *Science* **338** 1465–1469. (doi:10.1126/science.1227604)
- Xu D, Zhan Y, Qi Y, Cao B, Bai S, Xu W, Gambhir SS, Lee P, Sartor O, Flemington EK, et al. 2015 Androgen receptor splice variants dimerize to transactivate target genes. *Cancer Research* **75** 3663–3671. (doi:10.1158/0008-5472.CAN-15-0381)
- Yamamoto Y, Lin PJ, Beraldi E, Zhang F, Kawai Y, Leong J, Katsumi H, Fazli L, Fraser R, Cullis PR, et al. 2015 siRNA lipid nanoparticle potently silences clusterin and delays progression when combined with androgen receptor cotargeting in enzalutamide-resistant prostate cancer. *Clinical Cancer Research* **21** 4845–4855. (doi:10.1158/1078-0432.CCR-15-0866)
- Yeh S & Chang C 1996 Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. *PNAS* **93** 5517–5521. (doi:10.1073/pnas.93.11.5517)
- Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, Wang X, Wu L, Li J, Hu M, Gong Y, et al. 2010 An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell* **17** 443–454. (doi:10.1016/j.ccr.2010.03.018)
- Zaret KS & Carroll JS 2011 Pioneer transcription factors: establishing competence for gene expression. *Genes and Development* **25** 2227–2241. (doi:10.1101/gad.176826.111)
- Zeltser L, Desplan C & Heintz N 1996 Hoxb-13: a new Hox gene in a distant region of the HOXB cluster maintains colinearity. *Development* **122** 2475–2484.
- Zhan Y, Zhang G, Wang X, Qi Y, Bai S, Li D, Ma T, Sartor O, Flemington EK, Zhang H, et al. 2017 Interplay between cytoplasmic and nuclear androgen receptor splice variants mediates castration resistance. *Molecular Cancer Research* **15** 59–68. (doi:10.1158/1541-7786.MCR-16-0236)
- Zhang J, Zhang S, Murtha PE, Zhu W, Hou SS & Young CY 1997 Identification of two novel cis-elements in the promoter of the prostate-specific antigen gene that are required to enhance androgen receptor-mediated transactivation. *Nucleic Acids Research* **25** 3143–3150. (doi:10.1093/nar/25.15.3143)
- Zhao JC, Fong KW, Jin HJ, Yang YA, Kim J & Yu J 2016 FOXA1 acts upstream of GATA2 and AR in hormonal regulation of gene expression. *Oncogene* **35** 4335–4344. (doi:10.1038/onc.2015.496)
- Zong Y, Xin L, Goldstein AS, Lawson DA, Teitell MA & Witte ON 2009 ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. *PNAS* **106** 12465–12470. (doi:10.1073/pnas.0905931106)

Received in final form 24 May 2017

Accepted 30 May 2017

Accepted Preprint published online 31 May 2017