



Epigenetic Coregulation of Androgen Receptor Signaling

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Abstract

The androgen receptor (AR) is a ligand-activated transcription factor belonging to the nuclear receptor (NR) superfamily. As with other members of the NR family, transcriptional activity of the AR is regulated by interactions with coregulatory proteins, which either enhance (coactivators) or repress (corepressors) its transcriptional activity. AR associated coregulators are functionally diverse, but a large fraction are epigenetic histone and chromatin modifiers. Epigenetic coregulators are recruited to gene regulatory regions as part of multi-protein complexes, often acting in a dynamic and inter-dependent manner to remodel chromatin, thereby allowing or inhibiting the access of AR-associated transcriptional machinery to target genes; functional consequences being regulation of transcriptional output. Epigenetic modifiers, including those that function as AR coregulators, are frequently mutated or aberrantly expressed in prostate cancer and are implicated in disease progression. Some of these modifiers are being investigated as therapeutic targets in several cancer types and could potentially be

used to modulate aberrant AR activity in prostate cancer. In this chapter we will summarise the functional role of epigenetic coregulators in AR signalling, their dysregulation during prostate cancer progression and the current status of drugs targeting these enzymes.

Keywords

Epigenetics · Androgen receptor coregulators · Transcriptional regulation · Histone modifiers · Remodellers · Prostate cancer · Therapy

16.1 Introduction

The androgen receptor (AR) is a ligand-activated, DNA-binding transcription factor (TF), belonging to the nuclear receptor (NR) superfamily, that mediates responses to the androgenic (“male”) steroid hormones, most prominent of which are dihydrotestosterone and testosterone [1]. The AR-driven transcription program is a key determinant of organ morphogenesis during development and regulates functioning of the normal adult prostate, but is also the main driver of prostate carcinogenesis and disease progression [2, 3].

To activate its full transcriptional program, the AR must be bound and activated by ligands such as testosterone and dihydrotestosterone. AR

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binds to sequence-specific regulatory regions in the genome, where it interacts with accessory proteins called coregulators and transcriptional machinery to drive target gene expression [4]. Coregulators can be broadly defined as members of multi-protein complexes that associate directly or indirectly with transcription factors (TFs) and affect their output. These proteins are indispensable for TF functioning since they are rate-limiting factors for transcriptional activity that can either promote (coactivators) or suppress (corepressors) target gene expression, without affecting basal transcriptional levels [5–7]. Coregulators also dictate target gene specificity, with each coregulator associated with transcription of specific subsets of TF target genes [8]. The AR is associated with coregulators that encompass a wide variety of functional diversity and modes of action which can be broadly classified into the categories (not mutually exclusive) of epigenetic regulators, chaperones, transcriptional regulators, DNA repair proteins, cytoskeletal proteins and signal transducers, among others [9].

Epigenetic proteins are a key subset of coregulatory partners of AR and many other TFs as they are essential for transcriptional processes, regulating chromatin structure as well as accessibility. Alterations in epigenetic machinery proteins are frequent in prostate cancer and have been suggested to drive carcinogenesis and evolution of treatment resistance, as well as contribute to inter and/or intra tumoral heterogeneity [10, 11]. A number of these outcomes are the result of altered epigenetic coregulators disrupting AR signalling and such coregulators thus represent potential targets for therapeutics.

16.2 AR Structure and Coregulator Binding Interactions

The androgen receptor is a 919 amino acid protein (although size can vary due to the presence of a polymorphic polyglutamine tract) that can structurally be divided into four distinct domains: an N-terminal activation domain, (AF1), a DNA binding domain (DBD), a hinge region, and a ligand binding domain (LBD). The LBD contains the ligand binding pocket, a ligand-dependent activation domain (AF2) and binding function 3 (BF3) site [12–14]. A nuclear localization signal (NLS) is present in the DBD and hinge region [15]. In its unliganded form, the AR is largely localized in the cytoplasm in a heterocomplex with chaperones and immunophilins, which maintains it in a conformation conducive to ligand binding [16]. Upon binding to ligands, the AR dissociates from the chaperone complex and translocates to the nucleus where it homodimerizes and binds to palindromic dihexameric recognition sequences, termed androgen response elements (AREs), within regulatory regions (enhancers or promoters) of target genes [17, 18]. At AREs, the AR recruits and cooperates with other factors including coregulators; interactions between AR and partner protein domains leading to the assembly of large multiprotein complexes that are necessary for transcriptional regulation (Fig. 16.1A).

Coregulator recruitment by the AR is determined by type of ligand. Binding of an agonist within the LBD causes a conformational change, inducing formation of the AF2 hydrophobic binding cleft that recruits coactivators for receptor transactivation [19]. Interaction with AF2 is

Fig. 16.1 (continued) elements (AREs) and associates with coregulatory proteins and transcriptional machinery **(B)** Pioneer factors open compacted chromatin, allowing AR to access DNA **(C)** AR-associated ATP-dependent chromatin remodellers enhance chromatin accessibility

(D) Histone modifiers are recruited by the AR and modify surrounding histones **(E)** Epigenetic readers “read” histone marks and act as a link to recruit other protein complexes **(f)** Chromatin looping is required to link enhancers elements to gene promoters

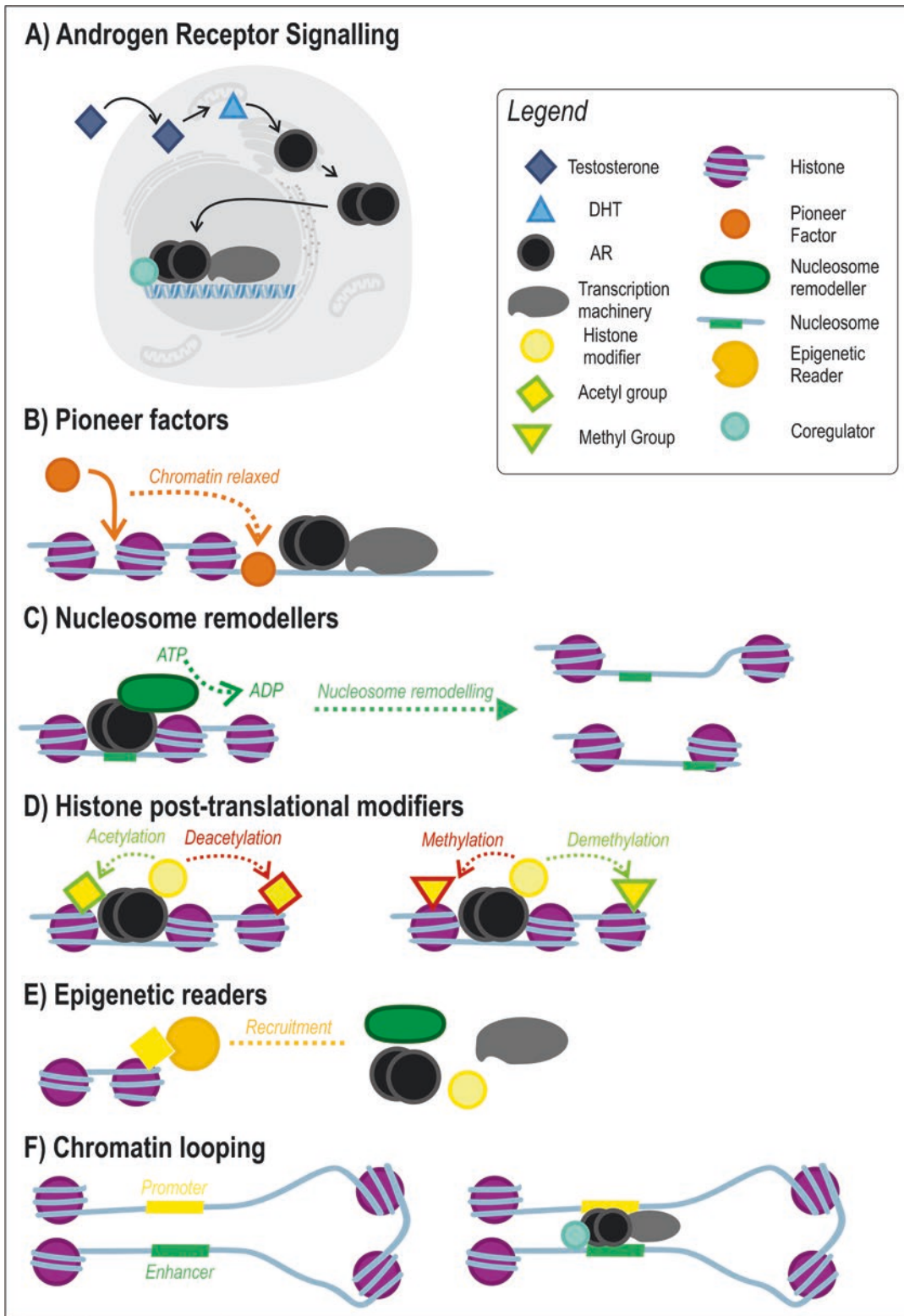


Fig. 16.1 Epigenetic regulation of androgen receptor signalling (A) Schematic diagram of AR mediated transcription in prostate cells. Testosterone is converted to

dihydrotestosterone (DHT) in prostate cells. DHT binds to the AR, promotes its dimerization and translocation to the nucleus. In the nucleus AR binds to androgen response

mediated by short, alpha-helical LXXLL or FXXLF motifs (L = leucine, F = phenylalanine, X = any amino acid) in coactivators, although the AR AF2 appears to preferentially bind to the FXXLF motif [20, 21]. Corepressors use extended LXXLL-like motifs, called corepressor nuclear receptor boxes (CoRNR boxes); these can interact with the AR following antagonist binding, which promotes conformational changes that accommodate this bulkier motif [22]. The AF2 coactivator binding site is blocked by this conformation of antagonist-bound AR [23]. Although these are the best-characterised modes of interaction, coregulator recruitment can also occur via interactions between the AR BF3 site, N-terminal AF1 or DBD and with regions other than the LXXLL/FXXLF motif in coregulators [13, 14, 19, 24].

16.3 AR-Coregulator Mediated Alteration of the Chromatin Landscape

Genomic DNA in eukaryotic nuclei is complexed around histone octamers to form nucleosomes, arrays of which are further coiled into heterochromatin. Chromatin is further compacted into higher order fibres, i.e. chromosomes [25, 26]. This compact organization of genomic DNA, by hindering indiscriminate access of transcription factors to binding sequences, facilitates tight regulation of gene expression. Key steps in gene transcription include dynamic reorganization of chromatin by transcription factor complexes, recruitment of basal transcription machinery, assembly of the preinitiation complex (PIC) at promoters and RNA polymerase activity [27].

The AR transcription complex assembled at target gene regulatory regions contains several coregulators that are modifiers of chromatin structure. This includes nucleosome remodellers, histone modifying/interacting proteins and mediators of chromatin looping (Fig. 16.1). Coregulators are initially recruited by interactions with the AR but subsequent coregulator recruitment is also dependent on targeting by and interplay with coregulators that are already part

of the complex [28]. The coregulator composition within an AR complex is likely to be both cell and target gene specific.

16.3.1 Pioneer Factors

Due to the aforementioned supercoiling of chromatin, most regions of genomic DNA are inaccessible for binding by transcription factors so their initial binding to recognition sequences is often facilitated by pioneer factors. These proteins have the unique ability to bind to and “relax” compacted chromatin, enabling access for other transcription factors and regulatory proteins (Fig. 16.1B) [29]. Pioneer factors have various mechanisms for de-compacting chromatin which include disrupting histone-DNA contacts to destabilize chromatin, evicting histones and recruiting chromatin modifiers [30, 31].

Pioneer factors collaborate with nuclear receptors to regulate distinct tissue-specific transcriptional programs [32]. The AR-associated pioneer factors FOXA1, HNF4 α and AP-2 α , for instance, regulate distinct AR cistromes (i.e., genome-wide AR binding sites) in the prostate, kidney and epididymis, respectively [33]. Besides FOXA1, other pioneer factors such as GATA2 and HOXB13 regulate the AR cistrome in normal as well as prostate cancer cells [34]. Indeed, these factors are critical for prostate cancer transformation and progression. Overexpression of FOXA1, for example, has been shown to increase AR chromatin binding to facilitate prostate cancer growth [35], while ectopic expression of FOXA1 and HOXB13 in a normal prostate epithelial cell line was shown to redistribute AR binding sites to resemble the pattern in prostate tumours [36]. Moreover, silencing of FOXA1 reprograms AR binding in prostate cancer [37]. Besides the full-length AR, both FOXA1 and GATA2 contribute to androgen deprivation therapy (ADT) resistant prostate cancer by acting as pioneer factors for the DNA binding of AR variants [38, 39]. In addition to enabling chromatin access directly, pioneer factors also facilitate recruitment of histone modifiers and remodellers for further chromatin decompaction [40, 41].

16.3.2 Nucleosome Remodellers

Besides binding to regions of the genome that are already open, AR also has the ability to further influence chromatin accessibility by regulating nucleosome occupancy at target enhancers [42–44]. To mediate these changes, the AR recruits a class of regulators that remodel chromatin using the energy from ATP hydrolysis to remove or reposition nucleosomes (Fig. 16.1C) [45]. AR activity is primarily coactivated by two subfamilies of ATP-dependent chromatin remodellers – the SWI/SNF and Chromodomain Helicase DNA-binding (CHD) proteins (Table 16.1).

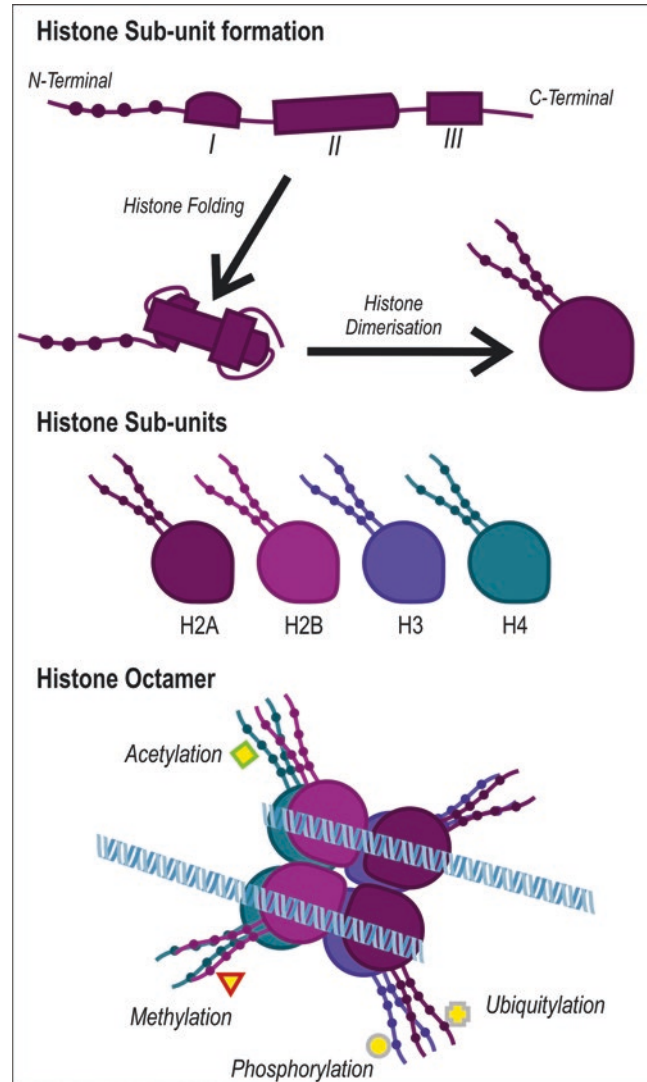
The SWI/SNF remodelling complex is a large complex comprised of 11–15 subunits (variable by context) including ATPases (BRG1, BRM) and core or associated factors that confer specificity [87, 88, 91]. Of these, BAF60a, BAF57 and SRG3 (a mouse homolog of human BAF155) interact with/coregulate the AR [87, 88, 91]. The ATPase present may also confer specificity, as BRG1 appears to regulate chromatin accessibility for a subset of AR target genes [92]. Most tumour types have mutations in one or more subunits of the SWI/SNF complex [93]. Unlike in other malignancies however, mutations of SWI/SNF subunits are uncommon in PCa but expression levels are often altered during disease progression [89, 94]. In benign and malignant prostate tissues BRG1 and BRM are reciprocally expressed, with increased BRG1/decreased BRM expression associated with cancer progression and metastasis [89]. Loss of BRM is also associated with prostatic hyperplasia and castration resistance in murine prostatic epithelia, but BRM-containing SWI/SNF complexes appears to be preferred for AR activity in cell line models, which is likely to be sustained by BRG1 or other remodelling complexes upon loss of BRM [95, 96]. SWI/SNF subunits can coactivate AR independently of the remodeller complex's ATPase function – SRG3, a core subunit of the mouse SWI/SNF complex, enhances AR transactivation even in the absence of both BRG1 and BRM [91].

The CHD family of remodellers consists of nine members (CHD 1-9) characterized by an N-terminal chromodomains and a central ATPase domain [97]. While some CHD proteins function as monomers, others are part of multiprotein complexes [98]. Members of the CHD family, have divergent functions, for example CHD8 acts as an AR coactivator and is upregulated in PCa whereas CHD1, which is associated with AR transcription at specific enhancers, is frequently deleted in PCa [90, 99]. Interestingly, although AR and CHD1 associate on chromatin and have significant overlap in their chromatin-bound interactome, they do not appear not to interact directly but may be bridged by overlapping interacting coregulators [99, 100].

16.3.3 Histone Post-translational Modifiers

Besides pioneer factors and nucleosome remodelling complexes, histone post-translational modifiers are also major regulators of chromatin accessibility (Fig. 16.1D). Histone octamers within nucleosomes consist of two copies of each of the canonical histones H3, H4, H2A and H2B [101]. Variant versions also exist, which can substitute for canonical histones and play essential roles during replication, gene regulation and repair [102]. Structurally, histone proteins contain a histone fold region and a tail region, with the fold regions responsible for formation of the octamer. Histone tails protrude out of the nucleosome and are targets for modifications that regulate chromatin structure (Fig. 16.2). Histone modifications occur on multiple residues within these N-terminal tails and also in the histone body [103]. Currently, at least 80 histone post translational modifications (PTMs) have been identified and include acetylation, methylation, phosphorylation, ubiquitylation, croonylation, succinylation, and sumoylation events. These modifications are frequently regulated in a coordinated manner with combined modifications governing regulatory events. Mechanisms by which histone PTMs modulate DNA accessibility differ: acetylation and phosphorylation for

Fig. 16.2 Histone structure and formation of octamers. Histone proteins are characterized by a tail region and a histone fold motif made up of a loop (I), central helix (II) and a short helix (III). The fold domain facilitates histone heterodimerization: two H2A-H2B dimers and a H3-H4 tetramer combine to form the octamer around which DNA is wound to form nucleosomes. Histone tails protruding from nucleosomes are targeted for post translational modifications



instance, alter the charge on histones thereby disrupting electrostatic interactions with DNA, whereas methylation enhances or disrupts interactions with chromatin binding factors [104].

Histone modifying/interacting proteins can be broadly classed as: writers, which deposit marks; those that remove marks, termed erasers; and readers that sense the modification and effect changes. AR associated histone writers and erasers modulate gene expression largely via changes in acetylation at lysine residues and methylation at lysine/arginine residues in histone tails (Table 16.1).

16.3.3.1 Histone Acetylases/Deacetylases

Histone acetylation is generally permissive of gene activation, and deacetylation is generally restrictive, with marks such as H3K27ac, H4K16ac, H3K9Ac and H3K14Ac enriched at active enhancers and/or promoters [105, 106]. Histone acetyl transferases (HATs), such as members of the NCOA/p160/Steroid Receptor Coactivator (SRC) family, p300/CBP and PCAF, are some of the earliest coregulators recruited by agonist activated AR [107]. Although p160/SRC proteins have weak histone acetylase activity

Table 16.1 AR-associated epigenetic coregulators and their published roles in prostate cancer

Coregulator	Function	Coregulator type	Role in PCa	References
Acetylases/Deacetylases				
CBP	Acetylates various residues on H1, H2, H3, H4	Coactivator	Upregulated in CRPC	[46, 47]
p300	Acetylates various residues on H1,H2,H3,H4; Acetylates AR	Coactivator	Upregulated in CRPC	[46, 47]
PCAF	H3K14, H3K9 acetylase	Coactivator	Oncogenic	[48]
KAT5/TIP60	Acetylates various residues on H2A, H3, H4; Acetylates AR	Coactivator	Upregulated in aggressive PCa	[49]
KAT7	H3K14/K23, H4K5/K8/K12 acetylase	Corepressor	Unknown	[50]
KAT8	H4K16 acetylase	Coactivator	Oncogenic	[51]
NCOA1/SRC1	H3, H4 acetylase	Coactivator	Oncogenic; Upregulated in PCa metastases	[52]
NCOA2/SRC2/TIF2	Weak histone acetylase activity	Coactivator	Overexpressed in recurrent PCa	[53]
NCOA3/SRC3/AIB1	H3, H4 acetylase	Coactivator	Oncogenic	[54]
HDAC1	Deacetylates H2A, H2B, H3 and H4	Corepressor	Upregulated in PCa	[55, 56]
HDAC2	Deacetylates H2A, H2B, H3 and H4	Corepressor	Upregulated in PCa	[56, 57]
HDAC3	Deacetylates H2A, H2B, H3 and H4	Corepressor	Upregulated in PCa	[56, 57]
HDAC7	Deacetylates H2A, H2B, H3 and H4	Corepressor	Unknown	[58]
SIRT1	Deacetylates H1 H2A, H2B, H3 and H4; Deacetylates AR	Corepressor	Upregulated in PCa	[59, 60]
NCOR1	Required for recruitment and/or activity of HDACs	Corepressor	Downregulated in PCa	[61, 62]
NCOR2/SMRT	Required for recruitment and/or activity of HDACs	Corepressor	Reduced expression associated with shorter disease-free survival	[62, 63]
Methylases/Demethylases				
CARM1	Asymmetric H3R16, R26 methylase	Coactivator	Upregulated in PCa	[64]
EZH2	H3 methylase	Coactivator	Oncogenic	[65]
PRMT1	H4R3 methylation	Coactivator	Oncogenic	[66]
NSD1	H3K36me2 methylase	Coactivator	Upregulated in metastatic PCa	[67, 68]
NSD2	H3K36me1/2 methylase	Coactivator	Pro-metastatic	[69, 70]
SET1	H3K4 methyltransferase	Coactivator	Oncogenic	[71]
SET9	H3K4me1,2 methylase, methylates AR	Coactivator	Oncogenic	[72, 73]
G9A	H3K9 methylation	Coactivator	Upregulated in PCa	[74]
KDM1A/LSD1	H3, H4 demethylase	Coactivator	Oncogenic	[75]
KDM3A/JMJD1A	H3K9me1/2 demethylase	Coactivator	Oncogenic	[76]
KDM4A/JMJD2A	H3K9me3 demethylase	Coactivator	Oncogenic, Upregulated in PCa	[24]
KDM4B/JMJD2B	H3K9 demethylase	Coactivator	Oncogenic, Upregulated in PCa	[77]

(continued)

Table 16.1 (continued)

Coregulator	Function	Coregulator type	Role in PCa	References
KDM4C/ JMJD2C	H3K9 demethylase	Coactivator	Oncogenic	[78]
KDM4D/ JMJD2D	H3K9me3 demethylase	Coactivator	Upregulated in PCa	[24]
KDM5B/ JARID1B	H3K4 demethylase	Coactivator	Upregulated in PCa	[79]
KDM7A	H3, H4 demethylase	Coactivator	Oncogenic; Upregulated in PCa	[80]
KMD8/ JMJD5	H3K36me2 demethylase	Coactivator	Upregulated in PCa	[81]
Epigenetic readers				
ING1b	H3K4me3 reader	Corepressor	Tumour suppressive; Downregulated in CRPC	[82]
ING2	H3K4me3 reader	Corepressor	Tumour suppressive	[83]
ING3	H3K4me3 reader	Coactivator	Oncogenic	[84]
TRIM24	H3K4, H3K23Ac reader	Coactivator	Oncogenic; Upregulated in CRPC	[85]
TDRD3	Asymmetric H3R17me2 and H4R3me2 reader	Coactivator	Unknown	[86]
Chromatin remodellers				
BAF57	SWI/SNF subunit	Coactivator	Oncogenic	[87]
BAF60a	SWI/SNF subunit	Coactivator	Unknown	[88]
BRG1	SWI/SNF subunit	Coactivator	Oncogenic	[89]
BRM	SWI/SNF subunit	Coactivator	Oncogenic	[89]
CHD8	CHD remodeller	Coactivator	Oncogenic	[90]

they have been proposed to act as a bridge, recruiting the more potent p300/CBP and PCAF HATs as well as the methyltransferase, coactivator-associated arginine methyltransferase 1 (CARM1) [107–109]. Members of the p160/SRC family are required for optimal expression of AR targets, with disruption of the interaction between AR and SRC-1 shown to selectively inhibit AR activity [110].

p300 and CBP are paralogous proteins that serve as critical coactivators of NR activity and are associated with the H3K18Ac/H3K27Ac active marks [111]. Both proteins interact with the AR, are recruited to regulatory regions of AR targets such as PSA and promote AR transcriptional activation [112]. p300, however appears to be dominant in the context of AR signalling, regulating many more AR targets than CBP [113, 114]. p300/CBP and the acetylation marks it deposited also appear to be necessary for recruitment of the SWI/SNF complex [28]. Several members of the evolutionarily conserved MYST

family of HATs also serve as AR coregulators: while Tip60/Kat5 and KAT8 are coactivators, KAT7 has been shown to repress AR activity [50, 115, 116].

Histone deacetylases (HDACs), which catalyse the removal of acetyl groups from histone and other proteins, are often recruited in cooperation with corepressors by antagonist bound AR: HDAC1 and 2, for instance, are recruited to AR target promoters along with the NCOR and SMRT corepressors in the presence of the AR antagonist bicalutamide [107]. Bicalutamide also represses AR transcriptional activity by recruiting the HDAC sirtuin1 (SIRT1), which in turn likely contributes to gene repression by deacetylating histone H3 at target promoters and enhancers [117]. Additionally, SIRT1 is able to reduce AR coactivation by p300 [59]. Some HDACs, such as HDAC1 and HDAC3, however, are required for transcription of AR activated genes as they facilitate coactivator and RNA PolII recruitment, suggesting that hyper-

acetylated chromatin may in some circumstances inhibit recruitment of these essential factors [118].

Both HATs and HDACs can also influence AR transcriptional activity independently of their histone modifying properties, by modifying the AR itself. The AR can be post-translationally modified by acetylation, phosphorylation, methylation and sumoylation; these affect protein stability, interactions with other proteins, localization and structure [119]. AR acetylation, carried out by p300, PCAF and TIP60/KAT5, occurs at lysine residues within a conserved KLKK motif in the AR hinge region and is critical for hormone induced activation, augmentation of AR activity, corepressor detachment and coactivator recruitment [55, 120, 121]. Conversely, HDACs serve to inhibit AR activity. This can happen either directly, such as when HDAC1 deacetylates AR to downregulate its activity, or indirectly by HDAC4 through a SUMOylation dependent mechanism [55, 122]. Interestingly, both TIP60/KAT5 and HDAC1 can co-exist in the same complex along with AR, potentially antagonizing each other's actions to control AR activity [55].

16.3.3.2 Histone Methylases/ Demethylases

The effects of histone methylation on gene activity are nuanced, and depend on the residue being modified and the number of methyl groups added [123]. Histone methylation is regulated by histone methyl transferases (HMTs) or demethylases (HDMs) which modify either arginine or lysine residues. Generally, methylation at H3K4, H3K36, H3K79 and H3R17 is associated with gene activation, while methylation at H3K9, H3K27 and H4K20 is associated with repression [124, 125].

As part of AR transcriptional complexes, HMTs/HDMs work in concert with other coregulators to affect modification at multiple histone residues, with different outcomes on AR activity. Examples of AR associated histone lysine modifiers include SET9, which activates transcription by methylating H3K4 at enhancers and TSS

regions, but prevents deposition of repressive dimethylation marks on H3K9 and JARID1B, which inhibits transcription via the removal of two and three methyl groups from H3K4 [72, 79]. Some methyltransferases are recruited to the AR transcriptional complex by HATs: e.g. CARM1, which methylates H3, requires the presence of the acetylases NCOA1/SRC1 or TIF2 to enhance AR activity [126]. On the other hand, methyltransferase activity can facilitate histone acetylation – the PRMT1 methyltransferase influences AR activity by methylating H4R3, which consequently results in H4 acetylation by p300, while SET9 is necessary for androgen induced recruitment of P/CAF [66, 72]. These interactions between different histone modification events also underscore the complexity of these regulatory events to finely tune and conditionally regulate transcription.

Other instances of interplay between histone modifiers include the demethylases KDM1A/LSD1 and JMJD2C, which act cooperatively to demethylate H3K9 resulting in activation of AR transcriptional activity [78]. KDM1A/LSD1, however, can also form a complex with the RCOR1/CoREST corepressor to demethylate H3K4, thereby turning off AR responsive enhancers [127, 128]. Histone acetylation and methylation at AREs can also involve crosstalk with phosphorylation. Protein kinase C-related kinase 1 (PRK1) acts in an androgen dependent manner to phosphorylate H3T11, which subsequently enhances demethylation of H3K9 by JMJD2C or LSD1 and acetylation of H3K9/K14, resulting in upregulation of AR activity [129]. PRK1 also promotes phosphorylation of H3T6 via protein kinase C beta I (PKC β I), which prevents KDM1A/LSD1 from demethylating H3K4 during AR-dependent gene activation [130].

Like acetylation, methylation of the AR protein at the KLKK (K = Lysine, L = Leucine) motif also affects its activity. Thus far, the HMT SET9 has been shown to directly methylate AR and enhance transcriptional activity. The demethylase KDM4B however can indirectly stabilize AR by interacting with it and potentially masking ubiquitin acceptor sites [73, 77].

16.3.4 Epigenetic Readers

Epigenetic marks established by modifiers are recognized and interpreted by effector proteins, called epigenetic readers, to modify chromatin structure. Reader proteins contain domains such as the plant homeodomain (PHD), Bromodomain and extra terminal (BET), Chromodomain (CHD), WD40 repeat (WDR) or Tudor domains which determine binding specificity, with the PHD, CHD and Tudor domains recognizing methylated lysine/arginine residues while BET proteins bind to acetylated lysines [131]. Reader proteins/domains link histone marks to other histone modifiers, or to remodelling, transcription, repair or other complexes (Fig. 16.1E). Of the PHD domain proteins the ING family, which binds to the H3K4 methylation mark and subsequently recruit HATs and HDACs, are AR coregulators [104]. While ING1 and 2 are corepressors, ING3 promotes AR transcriptional activity [82–84]. ING1 and ING2 are potentially recruited to AR transcriptional complexes through their reader activity. Their corepressor activity may relate to their role in recruiting the mSIN3A/HDAC repressor complex [82, 83]. While ING3 can target the AR coactivator HAT TIP60/Kat5 to H3K4me3 marks through its PHD reader activity, this mechanism does not appear to contribute to ING3 mediated AR transactivation. In this instance, ING3 has a cytoplasmic role scaffolding and increasing cytoplasmic TIP60 and AR interaction, subsequently enhancing receptor acetylation and nuclear translocation [84]. TDRD3, a Tudor domain reader of H3/H4 arginine marks, is an AR transcriptional coactivator likely functioning as a scaffolding molecule for assembly of protein complexes [86].

16.3.5 Chromatin Looping

Most AR enhancers, like enhancers generally, are located distal to promoters of target genes, which necessitates long-range interactions if they are to regulate gene expression. Regulatory regions for AR target genes were initially defined as located within 20–50 kb of the gene but an AR-bound

enhancer-target gene interaction spanning 650 kb has recently been reported [132, 133]. These interactions are mediated by AR and other proteins (including coregulators) bound at both sites and lead to the formation of chromatin loops (Fig. 16.1F). Several well-known AR targets, including PSA and TMPRSS2, are regulated by chromatin looping [134, 135]. The Mediator multi-subunit complex is a key regulator of gene expression through the formation of enhancer-promoter chromatin loops. The mediator complex bridges TFs at the enhancer with RNA pol II and preinitiation complex at promoters [136]. The MED1/TRAP220 subunit of this complex is a coactivator for the AR and other NRs, co-recruited along with the AR to AREs upon androgen stimulation [137]. MED1 depletion, or inhibition of its interaction with AR, leads to a reversal of androgen induced transcriptional changes in prostate cancer cell lines [137].

16.4 Dysregulated Expression and Function of Coregulators Promotes PCa Progression by Multiple Mechanisms

The AR plays a central role in prostate carcinogenesis and targeting it by ADT, with drugs such as enzalutamide, remains the standard of care for recurrent, advanced, and metastatic disease. ADT aims to block the action of the AR by either reducing levels of AR agonists (androgens) or by inhibiting the AR with antagonists (antiandrogens). While this is initially successful, patients usually progress to ADT-resistant prostate cancer (ADT-R-PC) within a few years [138]. ADT-R-PC is characterized by disease progression despite ADT, but the AR signalling axis remaining active in the majority of cases. Resistance to ADT via persistent AR signalling can occur via a number of AR signalling alterations, including AR amplifications, mutations, variants and coregulator associated mechanisms [138].

Dysregulated coregulator function and expression is a frequent feature of ADT-R-PC, suggesting an important role in disease progression and therapy resistance (Table 16.1) [8]. Indeed,

mechanisms by which epigenetic coregulators can induce aberrant AR signalling include (i) activating AR under low hormone conditions, (ii) post-translational modification of the AR or associated proteins (iii) facilitating interactions between AR and other factors and (iv) inducing expression of AR target genes in the absence of AR.

Taking the first such mechanism, coregulators can enable activation of the AR in the absence or low levels of agonists, thus escaping ADT-induced androgen blockade. TRIM24, for example, is a bromodomain containing histone acetyl reader that displays increasing expression in recurrent disease and as PCa progresses from primary to CRPC. Under low hormone conditions, TRIM24 can promote proliferation of PCa cells. This is attributed to its ability to regulate more AR responsive and cell cycle associated genes under hormone-starved compared to hormone-stimulated conditions [85]. Additionally, AR and TRIM24 coactivated genes are upregulated in CRPC and are predictive of recurrence [85]. This has been proposed to be a result of TRIM24 concomitantly binding to acetylated histones and AR, thus anchoring AR to chromatin, under androgen-depleted conditions [85]. As another example, increased levels of NCOA2/SRC-2/TIF2 in post-ADT recurrent PCa are proposed to activate AR signalling by increasing responsiveness of AR to lower affinity androgens [139]. Changes in SWI/SNF remodelling components are also able to contribute to disease progression and hormone independent disease. BAF57, which is upregulated with increasing tumour grade, enhances AR transactivation under androgen-depleted conditions [140].

Epigenetic coregulators that modify histones often also possess the ability to modify and stabilize AR and other proteins, thus contributing to androgen independence through this mechanism. For instance, overexpression of TIP60 in CRPC increases levels of acetylated AR, stabilizing it and consequently leading to increased localization in the nucleus despite the absence of androgens [141]. Increased expression of p300 has been demonstrated to be directly correlated with PCa proliferation, and to be a potential marker

predictive of aggressiveness and acquired ADT resistance. One mechanism for this might be its ability to acetylate and stabilize the histone demethylase JMJD1A, which results in enhanced AR activity and resistance to enzalutamide [142, 143]. In the case of the MED1 mediator subunit, phosphorylation by ERK or CDK7 is required for its coactivator activity [137, 144]. In enzalutamide resistant PCa cells, increased levels of phosphorylated MED1 are suggested to contribute to restored AR signalling [137].

Additionally, the ability of some coregulators to scaffold interactions between AR and other factors can indirectly promote aberrant AR activity. ING3 promotes activation of the AR by serving as a scaffold to increase interaction between AR and TIP60 [84]. This consequently leads to increased AR stability through acetylation, and activation of target gene transcription [84]. ING3 is potentially important for androgen independent growth since knockdown of this protein prevented cell growth under conditions that mimic ADT [84].

Finally, coregulators have been shown to compensate for loss of AR signalling by inducing expression of genes that drive PCa growth. Phosphorylated MED1 can induce expression of the AR target UBE2C through chromatin looping, in both AR positive and negative ADT-R-PC, to drive cell growth [145]. In another example, p300 promotes androgen-independent expression from the canonical AR target PSA promoter following long-term exposure of cells to IL-6, a cytokine elevated in patients with androgen-independent disease [146].

16.5 Therapeutic Targeting of AR Epigenetic Coregulators

Epigenetic enzymes have been of interest as therapeutic targets for the last few decades for several reasons, including: the reversible nature of epigenetic modifications; the tendency of epigenetic proteins to be differentially expressed in disease conditions; and the ability to inhibit these proteins using small molecule inhibitors [147]. Several small molecule inhibitors are available

for epigenetic coregulators that coactivate AR function, and have been tested preclinically or in early clinical trials for prostate cancer (Table 16.2). For the p300/CBP HAT, perhaps the most promising candidate so far is CCS1477 (Inobrodib), a potent and selective bromodomain inhibitor currently in Phase 1/2 trials for metastatic PCa and other solid tumours [46]. In vitro, this molecule inhibits PCa cell growth as well as signalling by AR or AR splice variants, and demonstrates anti-tumour activity in vivo [46]. Of the MYST family of HATs, inhibitors exist for KAT5,7 and 8 coactivators, although none have progressed beyond testing in cell line models. The NU9056 inhibitor for KAT5/TIP60 can affect AR levels and expression of PSA in PCa cell lines, potentially via inhibiting acetylase activity [49]. Furthermore, ADT-R-PC cell line models were more sensitive to NU9056 compared to androgen responsive lines, suggesting therapeutic potential [49]. Likewise, histone deacetylase inhibitors (HDACIs) are antiproliferative in preclinical models of prostate cancer. Inhibiting HDACs in PCa may seem counterintuitive given that HAT activity activates AR signalling, however HDACs are frequently upregulated in PCa and their inhibition has been found to suppress AR signalling. As mentioned earlier, HDAC 1 and 3 can activate AR transcription by coactivator and PolIII recruitment, an effect that is abrogated by HDACIs [118]. As another example, the LAQ824 HDACI represses AR activity by inducing acetylation of the HSP90

chaperone protein, which leads to its dissociation from the AR and subsequently AR degradation [153]. HDACIs also reduce AR mRNA and protein at the transcriptional level [118]. HDACIs are more effective in AR-positive prostate cell lines supporting the concept of these drugs acting in part through AR signalling. Indeed, synergistic effects have been observed in vitro on combining HDAC inhibitors with the anti-androgen bicalutamide and such combinations have been assessed in clinical trials [154, 155]. Bicalutamide has been shown to repress AR gene expression by recruiting HDACs [117], so synergistic effects observed on cotreatment with HDACIs are potentially mediated by other pathways. Histone methyltransferase and demethylase inhibitors are similarly promising candidates for therapeutic use with some, like the EZH2 inhibitor GSK126, showing synergistic effects with enzalutamide [151] (Table 16.2). Unlike acetylation, transcriptional effects of methylation tend to be residue specific, hence either methyltransferase or demethylase inhibitors may be required for inhibiting growth.

16.6 Conclusion

Androgen receptor coregulation by epigenetic enzymes is integral to its transcriptional activity. Epigenetic coregulators modulate AR transcriptional activity by diverse mechanisms, some of which have been adapted by prostate cancer cells

Table 16.2 Inhibitors targeting androgen receptor epigenetic coregulators in prostate cancer

Coregulator	Inhibitors	Testing status in prostate cancer	References
p300/CBP	CCS1477	Phase 1/2 clinical trial	[46]
HDACs	Panobinostat, Vorinostat	Phase 1/2 clinical trial (with Bicalutamide)	NCT00878436 NCT00589472
TIP60/KAT5	NU9056	Cell lines	[49]
CARM1	EZM2302	Cell lines	[148]
NSD2	MCTP-39	Cell lines, mouse xenografts	[69]
KDM7A	TC-E 5002	Cell lines	[80]
KDM1A/LSD1	INCB059872	Cell lines	[149]
KDM4A/B	NSC636819	Cell lines	[150]
EZH2	GSK126 PF-06821497	Cell lines Phase 1 clinical trial (for CRPC)	[151] NCT03460977
TRIM24	TRIM24-C34	Cell lines	[152]

to drive disease progression and/or therapy resistance. Epigenetic targets represent promising targets for PCa therapy, but only a few have currently made it to clinical testing. Further investigations into the role of these proteins in AR signalling have the potential for developing new therapies, particularly those that can work in combination with androgen pathway targeting therapy.

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