Chapter 5

Androgen Receptor as a Target for Epigenetic Therapy of Castration Resistant Prostate Cancer (CRPC)

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Introduction

Androgens and androgen receptor play key roles in the development of the prostate gland as well as the prostate cancer. Evidence from basic research and clinical studies indicates that androgen receptor and AR-dependent transcription remain activate in castration-resistant prostate cancer. Alterations in the androgen receptor signaling axis are central to castration-resistant prostate cancer progression, and have uncovered complex mechanisms by which AR and other components of the AR signaling axis affect, and are affected by different type of coregulatory proteins as well as epigenetic modifications such as DNA methylation and enzymes effecting histone modifications.

Persistent Androgen Receptor Signaling as a Driver of Castration-Resistant Prostate Cancer

Given the efficacy of androgen deprivation therapy (ADT) in suppressing circulating testosterone levels, it is believed that the castration-resistant prostate cancer progression (CRPC) is an AR-independent pathology, leading to its early designations as "hormone-refractory" or "androgen-independent" prostate cancer. Instead, many cancer have adapted to castrate conditions, through a number of possible mechanisms, still dependent on the AR signaling axis and in some cases still respond to androgens.

Thus, a major objective of prostate cancer research is to better understand the mechanisms underlying the transition from androgen-dependet to castration-resistant state.

The androgen deprivation therapy of locally advanced or metastatic PCa is achieved by blocking androgens (surgical or chemical castration) or directly antagonizing the AR by anti-androgens. In this stage, anti-androgens given to antagonize AR function behave as agonist to promote AR-mediated growth. The exact mechanism for development of this acquired agonist activity of anti-androgens in promoting AR function is largely unknown. Despite the development and approval of new and more potent androgen synthesis inhibitors and androgen receptor antagonists, castration-resistant or castration-recurrent prostate cancer continue to develop resistance to these therapeutics. In spite of ADT and the second-generation AR-targeting agent's enzalutamide (MDV3100) (1) or androgen synthesis inhibitor abiraterone acetate (2), the CRPC results within 2-3 years due to alterations in the androgen/AR signaling axis.

The androgen receptor is a member of the nuclear receptor superfamily of ligand-activated transcription factors (TFs). When not bound to its ligand, the AR protein is localized in the cytosol, sequestered, and stabilized by chaperone proteins. Upon binding of ligand, the AR undergoes a conformational change and translocates to the nucleus. In the nucleus, pioneer factors and histone

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modifiers prime the chromatin for transcription factors-binding, allowing the AR to bind as a dimer to androgen response elements (AREs) in the promoter or enhancer regions of its target genes. Promoter context and the relative and absolute level of coactivators and corepressors are key determinants of the resulting transcriptional activity of DNA-binding receptors. The PSA is the best known downstream target gene of the AR, which functions as the specific transcription factor that interact with its consensus binding elements. PSA expression is under the regulation of the AR, both in normal and cancerous prostate. However, in androgen-resistant state of the CRPC, cells continue to produce PSA at high levels possibly due to an AR that remains active despite low levels of its ligand, androgens, including androstenedione, but especially testosterone (T) and dihydrotestosterone (DHT). Androgens are important mediators of prostate cancer progression, as highlighted by the therapeutic targeting of the androgen receptor in men with metastatic PCa. Therefore, clinical treatments aimed at suppressing androgen receptor action include gonadotropin-releasing hormone analogues, gonadotropin-releasing hormone antagonists, and anti-androgens such as bicalutamide and enzalutamide.

Although the use of the biomarker prostate-specific antigen (PSA) has had an effect on the control of prostate cancer growth, the biological role of this enzyme in cancer progression is unclear. PSA (or gene name *KLK3*) is a member of the tissue kallikrein (KLK) family composed

of 15 genes (*KLK1-15*) and located in a tandem cluster on the long arm of human chromosome 19q13.3-13.4. Sequence identity between the *KLK* genes permits the categorization of this family into two subgroups; the first identified classical *KLK* genes (*KLK1*-3) sharing 65.8% (*KLK2*) and 61.5% (*KLK3*) sequence similarity with KLK1, and the new KLKs (*KLK4-15*) sharing about 34.9%-46.2% homology with *KLK1* (3). The KLK genes may share regulatory sequences, such as promoters and enhancers, like the similar AR-binding motifs found in the *KLK2* and *KLK3* sequences (4,5). Several AREs within these regulatory regions of *KLK2* and *KLK*3 genes are primarily responsible for transcriptional regulation by androgens. *KLK2* has two AREs; one at position -170 within its promoter (6) and another in the enhancer region -3819 to -3805 upstream from the transcription start site (7). The *KLK3* proximal promoter harbors two functional AREs (ARE-I and ARE-II) at positions -170 and -400 (8) and an additional ARE (ARE-III) in the far upstream enhancer region, which has a dramatic effect on *KLK3* transcription, in comparison to ARE-I and ARE-II (9). Similar regulatory promoter sequences of other kallikrein family members have not yet been identified.

Tissue PSA may promote androgen-resistant prostate cancer cell growth via enhancing NcoA4-induced AR transactivation without involving its protease activity (10).

NcoA4 is an androgen receptor coactivator and as coregulatory protein interacts with the AR in a ligand-dependent manner to enhance its transcriptional activity. It is highly probable that NcoA4 interacts with PSA and AR, possibly forming a tripartite complex.

Co-localization of PSA and NcoA4 was observed in the cytosol of high passage androgen-dependent LNCaP cells in the presence of androgen. While this complex was not detected at the promoter of androgen target genes, PSA enhances AR transcriptional activity in both LNCaP and CWR22rv1 cells, which was independent of its protease activity and was significantly inhibited by silencing NcoA4 expression. The consequent need for further clarification of the expression and/or distribution of NcoA4 splice isoforms revealed that amino acids 37-167 and 198-332 of the human NcoA4 sequences correspond to evolutionarily conserved regions referred to as ARA70 family domains I and II, respectively (11). The ARA70 has been characterized as having the capacity to enhance AR transcriptional activity in response to the anti-androgens hydroxyflutamide (HF) and 17β-estradiol (E2) in prostate cancer cells. (12). Consequently, NcoA4 may contribute to the acquired agonist activity of anti-androgens and plays an important role in making prostate cancer cells resistant to ADT (13).

At the castration-resistant stage, PCa tissue ARA70 and KLK2 expressions are both higher. One possible explanation is that with higher expression of ARA70, the ADT with the anti-androgen HF might enhance ARA70-induced AR transactivation, which may then result in the increased tissue KLK2 and PSA, both AR-regulated genes. The KLK2 combined with ARA70, and then promoted CRPC progress via the decrease of cell apoptosis via bax/bcl/caspase-3 signaling pathways as well as in the decrease of cell G1 arrest via modulation of p21/cdk2/cyclin D1 signalling pathways (14). Furthermore, the complex of KLK2 and ARA70 was necessary for CRPC cell survival. Neither KLK2 nor ARA70 downregulation respectively could repress CRPC cell survival.

Kallikrein 2 is co-expressed and co-localized with KLK3 (known as PSA), within the prostate tissue and are considered prostate-specific (15). Based on tissue expression patterns, tissue KLKs, such as KLK2 and PSA, are implicated in diverse physiologic processes. Although KLK2 was suggested to enhance the proliferation of castration-resistant cells, its role in tumor formation have not been fully elucidated. However, finding that KLK2 can promote PCa cell growth via interaction with the AR-ARA70 complex, might therefore represent a new pathophysiological role for KLK2.

Androgen Receptor-Binding Transcription Coregulatory Proteins

Transcriptional regulation is a complex process that requires many basal transcription factors for initiation and promoter-specific regulatory proteins (coactivators or corepressors) that either enhance or repress target gene expression. In the non-pathological state, the interplay of AR with its coregulators within the nucleus is tightly controlled. In the castration-resistant state, deregulation of this interplay is common manifested by increase expression of AR coactivators with a concomitant inhibition or loss of AR corepressors. Transcriptional functions of the AR, involving these coordinated actions of coactivators and corepressors, regulates chromatin modifying complexes and consequently genes transcription.

Ligand-activated AR recruits the p160 family of transcription coactivators that play a key role in facilitating aberrant AR signalling in CRPC. The p160 SRCs are known as coactivators for steroid receptors including AR, but activate a variety of other transcription factors such as NF-κB, TAT, or AR-1. The C- terminus of the SRCs contains two activation domains which can recruit CREB- binding protein (CBP) and p300, as well as other coactivators and histone modifiers, such as CARM-1 (coactivator-associated arginine methyltransferase) and PRMT1 (pro-

tein arginine methyltransferase 1). Therefore, the p160 steroid receptor coactivators, including steroid receptor coactivator-1 (SRC-1, or NCOA1), steroid receptor coactivator-2 (SRC-2, or NCOA2, TIF2), and steroid receptor coactivator-3 (SRC-3, or NCOA3) possess histone acetyltransferase activity (HAT) and complex with CBP/p300 on DNA bound-ligand nuclear receptors. Consequently, the main function of the CBP/p300 coactivator complex is the regulation of transcription through remodelling of chromatin by acetylating histones.

Overexpression of SRC-1, SRC-2, and SRC-3 mRNA has been observed in patients with primary PCa as well as patients treated with ADT. This is emphasized through the observation that the p160 SRCs have been shown to coactivate the AR not only with androgens but even in the presence of partial agonists (16). However, the most studied SRCs in PCa is SRC-2. In mouse model, SRC-2 has been induced upon castration and, in this model, has been demonstrated to be critical for the development of CRPC (17). Although SRC-3 has been shown to be upregulated at the mRNA level in patients with PCa, the number of patients with increased mRNA is unimpressive when compared with SRC-1 or SRC-2. This finding suggests that SRC-3 may be extensively regulated at the posttranslational level in PCa. For example, another negative regulator of AR activity is the E3 ubiquitin ligase adaptor speckle-type poxvirus and zinc finger (POZ) domain protein (SPOP) that interacts with SRC-3 and promotes

its culling 3-dependent ubiquitination and degradation of several protein substrates, including the androgen receptor coactivator SRC-3 (18,19). Interestingly, somatic heterozygous missense mutations in the SPOP substrate-binding cleft recently were identified in up 15% of PCas, but their contribution to PCa pathophysiology remains unknown. All SPOP mutants identified to date lack the capacity to interact with SRC-3 [18] or AR itself [19], resulting in the stabilization of these substrates.

Forkhead Box A1 (FOXA1) is not a classic transcriptional coactivator, and together with GATA-2 belongs to two classes of proteins that have been identified as pioneer factors for AR recruitment to chromatin (20,21,22). Moreover, it has been proposed that FOXA1 is not required for AR-chromatin interaction at AREs but is instrumental in recruiting AR to low-affinity half-AREs by opening local chromatin (23). In the presence of high levels of FOXA1, these open chromatin regions serve as reservoirs that retain AR via abundant half-AREs and decrease availability of AR for full-ARE sites. Thus, suppressing FOXA1 can release AR to bind new full-AREs across the genome, resulting in reprogramming of the AR and AR-dependent gene expression, even in absence of androgens (24,25). Consequently, FOXA1 downregulation activates a castration-resistant AR transcription (26), while lower FoxA1 levels are significantly associated with increased metastatic potential and poor prognosis (27). GATA-2 is another pioneer factor that regulates AR function and, unlike

FOXA1, also promotes AR expression. GATA-2 silencing resulted in decrease in both AR mRNA and protein levels in androgen-free and R1881-treated cells (28), diminishing the expression of not only full-length AR but also alternatively spliced AR variants (ARVs) (29).

As ARVs have emerged as a mechanism of CRPC resistance to androgen synthesis inhibitors or AR antagonists, the finding that inhibition of GATA-2 may not only target the full-length AR signalling axis but also decreases the expression and function of ARVs is critical (30).

Alterations to AR corepressors also play a key role in CRPC. Loss activity of the key nuclear receptor corepressors SMRT (silencing mediator of retinoid and thyroid hormone receptors, also known as N-CoR2) and N-CoR1 (nuclear receptor corepressor) is relatively common in primary PCa and enriched in CRPC. N-CoRs (N-CoR1 and N-CoR2) corepressors compete with key AR coactivators, such as p300, CREB-binding protein (CBP)/p300, or SRCs (p160) coactivators, for binding to the ligand-activated receptor, thereby inhibiting its transcriptional activity (31). Consequently, the subsequent loss of these factors facilitates AR signalling in malignant tissues.

Methylation as an Epigenetic Repressive Mechanism with Silencing Impact on Gene Transcription

Transcriptional activities of the androgen receptor (AR) are precisely modulated at multiple levels, including coregulatory proteins as well as post- translational epigenetic modifications. Androgen receptor itself has no intrinsic enzymatic activity; instead, it serves as a scaffold recruiting various protein machineries that can modify chromatin and result in active transcription. Epigenetic modifications are complex independently working mechanisms that have recently became important factors of cancer development and progression. It has become increasingly apparent that all human cancers have epigenetic abnormalities that collaborate with genetic changes to drive tumor progression stages.

Epigenetic mechanisms are exerted in both global changes in chromatin and in localized changes affecting transcription factor binding to specific gene promoter. Tumor-specific alterations in the epigenetic modified chromatin are attributed to altered expression of chromatin-modifying enzymes such as non- histone substrates for histone methylases and demethylases. Because many of these targets are transcriptional regulators with crucial roles in proliferation control, methylation may function as

important regulator of histone-modifying enzymes affecting in this way cancer development.

Research in cancer epigenetics has been largely focused on DNA methylation although acetylation and phosphorylation appear to be integrated epigenetic process. DNA methylation is necessary for long-term silencing of imprinted genes and repetitive elements. DNA hypomethylation in conjunction with local promoter-specific hypermethylation is implicated in numerous of human diseases associated with chromosomal instability syndromes to cancer.

Site-specific regulation of DNA methylation is mediated by DNA-binding transcription factors that interact with specific promoter or enhancer regions. Concentrated on study of the DNA methylation itself is certainly important, but seems more fruitful relationship between DNA methylation and binding transcription factors.

Apart from influencing local chromatin structure, histone modifications are also recognized by specific adaptor proteins which in turn recruit protein complexes and thereby affect gene regulation. Histone methyl marks such as H3K4me3, K3K9ac, and H3K14ac are associated with actively transcribed genes and some other modifications, H3K27me3 and H3K9me3, are enriched within repressed regions. The methyl marks themselves are required for the above configurations, as they provide a surface recognized by specific adaptor proteins with a "reader" function.

CpG islands are characterized their GC-rich base composition and high density of CpG dinucleotides. Most human promoters are embedded within CpG islands that lack DNA methylation and coincide with sites H3K4me3 independently of transcriptional activity. Non-methylated CpG islands reach chromatin is enriched for histone modifications associated with actively transcribed genes (*i.e.* acetylated H3, H3K4me3 and H3K4me2) compared with bulk chromatin. In contrast, CpG islands reach chromatin is depleted for marks not found at active promoters: H3K36me3, H3K9me3, H3K27me3 and H4K20me3. In spite of these correlations, the functional significance of non- methylated CpG island sequences with respect to chromatin structure and transcription is unknown. A subset of promoters associated with both activating (H3K4me3) and repressive (H3K27me3) histone is described as a "bivalent" modification (32,33). In accordance with this, several thousands of the K3K4me3- enriched promoters in pluripotent cells contain the H3K27me3 repressive histone mark (34).

Polycomb group (PcG) proteins were discovered as epigenetic silencing genes during embryogenesis, many of them have been implicated in development and differentiation (35). The PcG proteins are transcriptional repressors (36) that function in two distinct Polycomb repressive complexes – PRC1 and PRC2 (or human homolog ESC-E(Z) complex). The PRC2 contains three components: enhancer of zeste 2 (EZH2) or its close homolog EZH1, embryonic ectoderm development (EED) and suppres-

sor of zeste 12 (Suz12). As components of PRC2, EZH2 and EZH1 can catalyze mono-, di- and trimethylation of H3K27 (37). Most of the cancer associated effects of EZH2 induction can be explained just by its transcriptional repression function (38). Therefore, the EZH2 is one of the best studied histone methyltransferase in cancer research.

Epigenetic silencing in cancer cells is mediated by at least two distinct histone modifications, PcG-based H3K27me3 and H3K9me2. The relationship between DNA hypermethylation and these histone modifications is not completely understood. One possibility is that the EZH2 as a component of the PRC2 complex with histone methyltransferases function can interact with histone deacetylases (HDACs) as well as with several DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B). The DNMT1 target is a newly formed DNA strand during replication. DNMT3A and DNMT3B are *de novo* DNA methyltransferases, whose role is to maintain new methylation pattern that forms due to formation of the cancer. While HDACs activity and histone deacetylation are required for EZH2-mediated H3K27 methylation, this methylation in turn is thought to predispose DNA to methylation by DNMTs (39).

The repressive effects of DNA methylation involve several mechanisms (40,41). DNA methylation can prevent transcriptional regulators from recognizing their cognate targets, or can recruit regulators that specifically

bind methylated DNA. CpG islands may be protected prior to their methylation by binding of TFs. Proteins CFP1 (CXXC finger protein 1) and KDM2 containing zinc finger domains, thus bind to unmethylated CpG dinucleotides regardless of the surrounding sequence (42). The zinc finger motifs are typical for transcription factors and proteins regulating gene expression. Moreover, the transcription factor binds CFP1 methyltransferase in histone H3K4 site and thereby protects bound sections from DNA methylation (43). Both transcription factors - CFP1 and KDM2 (histone lysine demethylase 2A) are associated with enzymatic activities that modulate specific methylation patterns of the histones. This is one of the ambiguities whose clarification could help to more accurate view of the CpG island importance: proteins binding to unmethylated DNA segments indicate changes of chromatin environment, which in turn affects a level of the gene regulation.

While DNA methyltransferases catalyze methylation of cytosine bases in CpG poor regions, CpG islands in promoter remain hypomethylated. Hypermethylatin of CpGs in the promoter or regulatory sequence of a gene is considered as a non-mutational mechanism that can cause repression of transcription of genes associated with cancer. In contrast to PcG-mediated repression, the DNA methylation is considered a more permanent silencing mechanism. However, not all PcG-targeted genes implicated in cancer are marked with DNA methylation. For

example, although only 5% of promoters in prostate cancer cells were methylated, downregulation of the EZH2 histone methyltransferase restored expression of the H3K27me3 alone or in synergy with histone deacetylase inhibition and with no effect on the expression of genes silenced by DNA hypermethylation (44).

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References

1. RScher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012; 367: 1187-1197.
2. deBono JS, Logothetis CJ, Molina A, Fizazi K, North S, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011; 364: 1995-2005.
3. Harvey TJ, Hooper JD, Myers SA, Stephenson SA, Ashworth LK, et al. Tissue-specific expressions patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. J Biol Chem. 2000; 275: 37397-37406.

4. Lawrence MG, Stephens CR, Need EF, Lai J, Buchanan G, et al. Long terminal repeats act as androgen-responsive enhancers for the PSA-kallikrein locus. Endocrinology. 2012; 153: 3199-3210.
5. Lawrence MG, Lai J, Clements JA. Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. Endocr Rev. 2010; 31: 407-446.
6. Murtha P, Tindall DJ, Young CY. Androgen induction of a human prostate- specific kallikrein, hKLK2: characterization of an androgen response element in the 5´promoter region of the gene. Biochemistry. 1993; 32: 6495-6464.
7. Yu DC, Sakamoto GT, Henderson DR. Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy. Cancer Res. 1999; 59: 1498-1504.
8. Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem. 1996; 271: 6379-6388.
9. Cleutjens KB, van der Korput HA, van Eekelen

CC, van Rooij HC, Faber PW, et al. An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol Endocrinol. 1997; 11: 148-161.

10. Niu Y, Yeh S, Miyamoto H, Li G, Altuwaijri S, et al. Tissue prostate specific antigen (PSA) facilitates refractory prostate tumor progression via enhancing ARA70-regulated androgen receptor transactivation. Cancer Res. 2008; 68: 7110-7119.
11. Kollara A, Ringuette MJ, Brown TJ. Dynamic distribution of nuclear coactivator 4 during mitosis: association with mitotic apparatus and midbodies. PLoS ONE. 2011, 6: e22257.
12. Yeh, S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Sci USA. 1996; 93: 5517-5521.
13. Rahman MM, Miyamoto H, Takatera H, Yeh S, Altuwaijri S, et al. Reducing the agonist activity of antiandrogens by a dominant-negative androgen receptor coregulatory ARA70 in prostate cancer cells. J Biol Chem. 2003; 278: 19619-19626.
14. Shang Z, Niu Y, Cai Q, Chen J, Tian J, et al. Human kallikrein 2 (KLK2) promotes prostate cancer cell growth via function as a modulator to promote

the ARA70-enhanced androgen receptor transactivation. Tumor Biol. 2014; 35: 1881-1890.

15. Mize GJ, Wang W, Takayama TK. Prostate-specific kallikreins-2 and -4 enhance the proliferation of DU-145 prostate cancer cells through protease-activated receptors-1 and -2. Mol Cancer Res. 2008; 6: 1043-1051.
16. Agoulnik IU, Weigel NL. Coactivator selective regulation of androgen receptor activity. Steroids. 2009; 74: 669-674.
17. Qin J, Lee H-J, Wu S-P, Lin S-C, Lanz RB, et al. Androgen deprivation- induced NCoA2 promotes metastatic and castration-resistant prostate cancer. J Clin Invest. 2014; 124: 5013-5026.
18. Geng C, Rajapakshe K, Shah SS, Shou J, Eedunuri VK. Androgen receptor is the key transcriptional mediator of the tumor suppressor SPOP in prostate cancer. Cancer Res. 2014; 74: 5631-5643.
19. Geng C, He B, Xu L, Barbieri CE, Eedunuri VK, et al. Prostate cancer- associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnoever. Proc Natl Acad Sci U S A. 2013; 110: 6997-7002.
20. Wang Q, Li W, Zhang Y, Yuan X, Xu K, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009; 138: 245-256.

21. Gao N, Zhang J, Rao MA, Case TC, Mirosevich J, et al. The role of hepatocyte nuclear factor-3 alpha (Forkhead Box A1) and androgen receptor in transcriptional regulation of prostatic genes. Mol Endocrinol. 2003; 17: 1484-1507.

22. Cirillo LA, Lin FR, Cuesta I, Friedman D, Jamik M. et al. Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. Mol Cell. 2002; 9: 279-289.

23. Jin H-J, Zhao JC, Wu L, Kim J, Yu J. Cooperativity and equilibrium with FOXA1 define the androgen receptor transcriptional program. Nat Commun. 2014; 5: 1-14.

24. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, et al. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature. 2011; 474: 390-394.

25. Wu D, Sunkel B, Chen Z, Liu X, Ye Z, et al. Three-tiered role of the pioneer factor GATA2 in promoting androgen-dependent gene expression in prostate cancer. Nucleic Acids Res. 2014; 42: 3607-3622.

26. He B, Lanz RB, Fiskus W, Geng C, Yi P, et al. GATA2 facilitates steroid receptor coactivator recruitment to the androgen receptor complex. Proc Natl Acad Sci U S A. 2014; 111: 18261-18266.

27. Foley C, Mitsiades N. Moving beyond the androgen receptor (AR): Targeting AR-interacting proteins to treat prostate cancer. Horm Canc. 2016; 7:84-103.

28. Foley C, Mitsiades N. Moving beyond the androgen receptor (AR): targeting AR-interacting proteins to treat prostate cancer. Horm Cancer. 2016; 7: 84-103.

29. Yoon HG, Wong J. The corepressors silencing mediator of retinoid and thyroid hormone receptor and nuclear receptor corepressor are involved in agonist- and antagonist-regulated transcription by androgen receptor. Mol Endocrinol. 2006; 20: 1048-1060.

30. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med. 2016; 22: 369-378.

31. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015; 161: 1215-1228.

32. Azuara V, Perry P, Sauer S, Spivakov M, Jørgensen HF. Chromatin signatures of pluripotent cell lines. Nat Cell Biol. 2006; 8:532-538.

33. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006; 125: 315-326.

34. Harikumar A, Meshorer E. Chromatin remodeling and bivalent histone modifications in embryonic stem cells. EMBO Rep. 20105; 16: 1609-1619.

35. Orlando V. Polycomb, epigenomes, and control of cell identity. Cell. 2003; 112: 599-606.

36. Ringrose L, Paro R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet. 2004; 38: 413-443.

37. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol. 2013; 20: 1147-1155.

38. Sarris M, Nikolaou K, Talianidis I. Context-specific regulation of cancer epigenomes by histone and transcription factor methylation. Oncogene. 2014; 33: 1207-1217.

39. Yang YA, Yu J. EZH2, an epigenetic driver of prostate cancer. Protein Cell. 2013; 4: 331-341.

40. Blattler A, Farnham PJ. Cross-talk between site-specific transcription factors and DNA methylation states. J Biol Chem. 2013; 288: 3428-3494.

41. Buck-Koehntop BA, Defossez PA. On how mammalian transcription factors recognize methylated DNA. Epigenetics. 2013; 8: 131-137.

42. Blackledge NP, Thomson JP, Skene PJ. CpG island chromatin is shaped by recruitment of ZF-CxxC proteins. Cold Spring Harb Perspect Biol. 2013; 5: a018648.

43. Thomson JP, Skene PJ, Selfridge J, Clouaire T, Guy J, et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. Nature. 2010; 464: 1082-1086.

44. Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet. 2008; 40: 741-750.

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