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INVITED REVIEW

Prostate Cancer

Epigenetic regulation of prostate cancer: the theories and the clinical implications

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Epigenetics is the main mechanism that controls transcription of specific genes with no changes in the underlying DNA sequences. Epigenetic alterations lead to abnormal gene expression patterns that contribute to carcinogenesis and persist throughout disease progression. Because of the reversible nature, epigenetic modifications emerge as promising anticancer drug targets. Several compounds have been developed to reverse the aberrant activities of enzymes involved in epigenetic regulation, and some of them show encouraging results in both preclinical and clinical studies. In this article, we comprehensively review the up-to-date roles of epigenetics in the development and progression of prostate cancer. We especially focus on three epigenetic mechanisms: DNA methylation, histone modifications, and noncoding RNAs. We elaborate on current models/theories that explain the necessity of these epigenetic programs in driving the malignant phenotypes of prostate cancer cells. In particular, we elucidate how certain epigenetic regulators crosstalk with critical biological pathways, such as androgen receptor (AR) signaling, and how the cooperation dynamically controls cancer-oriented transcriptional profiles. Restoration of a "normal" epigenetic landscape holds promise as a cure for prostate cancer, so we concluded by highlighting particular epigenetic modifications as diagnostic and prognostic biomarkers or new therapeutic targets for treatment of the disease.

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INTRODUCTION

The last two decades have witnessed a huge advance in our understanding of prostate cancer (PCa), which leads to the development of new therapeutic modalities including chemotherapy, immunotherapy, and novel hormonal reagents. Although better treatments have significantly improved life expectancy in patients, the disease remains the most common nondermatologic type of cancer and the third leading cause of cancer mortality in men in the United States.[1] As a highly heterogeneous disease, PCa is driven by both genetic and nongenetic causes. Common genetic changes with well-defined roles in the disease include loss of heterozygosity (LOH) of *p53*[2] and phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*)[3] genes, fusion of transmembrane protease, serine 2 (*TMPRSS2*) promoter with external transcribed spacer (*ETS*) transcription factor genes,[4] and mutations of speckle-type POZ protein (*SPOP*) gene.[5] However, not all cases of prostate tumorigenesis can be explained by definitive driving genomic alterations. It is quite likely that other biological events precede and enforce the malignant transformation. Epigenetic alteration is one of such candidates.

Epigenetics refers to any biological processes that modulate gene expression and subsequently control cell fate without affecting the actual DNA sequences.[6] The topics that are currently covered in studies of epigenetics include DNA methylation, histone modifications, chromatin remodeling, and noncoding RNA processing. It has been shown that numerous epigenetic alterations appear to be highly recurrent, and sometimes nearly universal, in PCa. These alterations reinforce the establishment of a context-specific transcriptional profile that favors self-renewal, survival, and invasion of PCa cells. It has been demonstrated that accumulation of epigenetic aberrations eventually causes genetic or genomic instability. On the other hand, several genes encoding the enzymes that shape the epigenetic landscape are found mutated in PCa. Therefore, genetic mutations and epigenetic aberrations contribute individually and cooperatively to the pathogenesis and progression of PCa. In this review, we will spotlight functions of three epigenetic programs, *i.e.*, DNA methylation, histone modifications, and noncoding RNAs, all of which have been comprehensively studied in prostate carcinogenesis and tumor progression. The purpose of this article is to systematically overview the evidences that support the theoretical foundation for epigenetic diagnosis, prognosis, and therapy in PCa.

DNA METHYLATION

DNA methylation is a chemical reaction by which a methyl ($-CH_3$) group is covalently attached to either cytosine or adenine of DNA molecules.[7] This modification is catalyzed primarily by three members of DNA methyltransferase family: DNMT1, DNMT3A, and DNMT3B.[8] It can be removed either passively on daughter strand after several rounds of DNA replication or actively by multiple enzymes like Ten-eleven

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translocation (TET) family proteins with the assistance of base excision repair (BER) pathway.[9] DNA methylation is one of the most critical epigenetic regulatory mechanisms affecting gene expression potentials. High levels of methylation at promoter regions or around the transcription start sites (TSS) usually lead to transcriptional silencing.[10] Several theories have been put forward to explain the correlation of promoter methylation with low gene expression.[11] First, proteins that specifically bind to methylated DNA block chromatin accessibility of transcription factors required for gene induction. Alternatively, such transcription factors no longer recognize regulatory elements of target genes because of the modification. Besides, DNA methylation establishes a repressive and closed chromatin structure, as suggested by the insensitivity of the modified chromatin to nuclease digestion and significantly less acetylation of histone proteins assembled on it. On the other hand, DNA methylation is found to be enriched in intragenic regions as well, which has been shown to play a regulatory role in transcriptional elongation and alternative splicing.[12] Particularly, gene bodies of highly transcribed genes are heavily methylated, and the methylation intensity is positively correlated with the levels of expression.[13] This indicates a universal function of intragenic methylation in transcriptional activation. Emerging evidence suggests that the histone mark H3K36 trimethylation (H3K36me3), which is instructive for transactivation, facilitates gene body DNA methylation by docking the functional PWWP domain of DNMT3B and recruiting the DNA methyltransferase to targeted genomic sites.[14,15] The PWWP domain is a 100-150 amino acid module that contains a highly conserved Pro-Trp-Trp-Pro motif. It functions to associate with chromatin and therefore is often found in many DNA-binding proteins. Spatial selectivity of DNA methylation and multiple mechanisms of action all indicate how critical this epigenetic machinery can be in terms of gene expression regulation; thus, any misregulation of this precise machinery may result in human diseases and disorders, such as cancers.

Changes in DNA methylation landscape, either globally or locus specifically, have been indicated as one of the most recurrent events in advanced PCa (**Figure 1**).[16] This supports a causal role of these specific somatic alterations in driving neoplastic phenotypes and aggressive evolution of the disease. In mammals, DNA methylation predominantly occurs in the context of CpG dinucleotide, and merely 1% of human genome contains this dinucleotide due to spontaneous deamination of methylated CpG to TpG over time. The only exception of this global CpG under-representation is specific genomic regions enriched for GC sequences, which is termed CpG islands. In normal cells, promoter CpG islands are predominantly unmethylated. However, some of them get hypermethylated when cells become transformed or malignant. Consistent with its presumed function, hypermethylation of promoters coincides with transcriptional repression of downstream target genes, most of which are supposed to act as tumor suppressors.

Glutathione S-transferase pi (*GSTP1*) is one of the genes that are heavily methylated at the promoter region and concomitantly shows loss of expression in prostate tumors. *GSTP1* hypermethylation occurs in more than 90% of the clinical cases and presents at all stages during PCa progression.[17] This finding has been repeatedly confirmed in independent samples by separate research groups,[18,19] which best exemplifies high recurrent rate of DNA hypermethylation at particular genomic loci. Hundreds of additional genes have been reported to contain hypermethylated promoters, such as O^6-methylguanine DNA methyltransferase (*MGMT*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), death-associated protein kinase (*DAPK*), and tissue inhibitors of metalloproteinases (*TIMPS*), just to name a few.[20–22] These genes exert dynamic biological functions and are involved in a number of pivotal cellular pathways such as hormonal response, tumor cell invasion/metastasis, cell cycle control, apoptosis, and DNA damage repair. Nowadays, a clinical inspection called ConfirmMDX (MDxHealth) assesses methylation signals at promoters of three genes (*GSTP1*, adenomatous polyposis coli [*APC*], and Ras association domain family 1 [*RASSF1*]), which independently predicts PCa incidence relative to traditional parameters such as prostate-specific antigen (PSA) levels and digital rectal examination results. This test represents the first epigenetics-based diagnostic assay, and it helps minimize false-negative cases by inspecting prostate biopsies that initially show histopathologically cancer-free. Therefore, this testing solution improves patient risk stratification and avoids unnecessary screening procedures. The National Comprehensive Cancer Network (NCCN) has included this analysis in the guidelines for early PCa detection to ensure more efficient diagnoses and to improve clinical outcomes.[23,24] Inspired by the proof of concept, scientists strive to develop new approaches utilizing DNA hypermethylation for PCa screening, and ProCam is one of such investigational tests. This assay evaluates the epigenetic modifications in *GSTP1*, retinoic acid receptor cDNA probe (*RAR2*), as well as *APC*, and preliminary results are very encouraging.[25] More importantly, ProCam uses urine samples instead of needle biopsies, so it may be a noninvasive algorithm for the detection of PCa.

In contrast to hypermethylation-mediated gene silencing, hypomethylation, which means demethylation of normally methylated DNA, contributes to gene overexpression. A distinctive feature of DNA hypomethylation in many malignancies is that loss of DNA methylation seems to be a genome-wide phenomenon rather than at individual genes. In 1987, Bedford and van Helden reported that

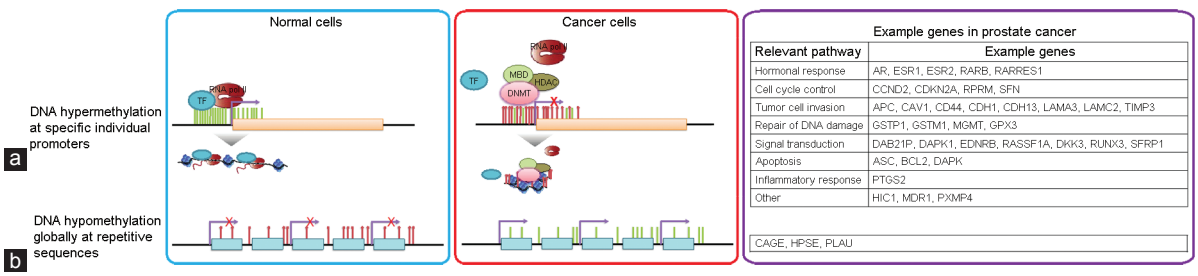

Figure 1: Alterations in DNA methylation patterns change expression of certain genes in PCa. Both DNA (**a**) hypermethylation and (**b**) hypomethylation are found in cancer cells. Effects of these changes in DNA methylation profiles on gene expression, chromatin structure, or transcriptional regulatory network are demonstrated as what the zoomed-in areas depicted. Genes that are transcriptionally modulated by each specific mechanism of DNA methylation in PCa are listed, categorized by the biological functions each representative gene may be involved in. PCa: prostate cancer; green bars: sites in unmethylated states; red bars: methylated sites.

the overall content of methylated DNA was significantly lower in metastatic prostate tumors compared to normal or benign hyperplastic tissues. This is the first study demonstrating a correlation between hypomethylation and metastatic potential of PCa.[26] Ever since then, plenty of work indicates that global hypomethylation becomes more prominent as prostate tumors progress to advanced stages.[27,28] For example, urokinase plasminogen activator (*PLAU*) is known to promote aggressive phenotypes of PCa cells.[29,30] Its expression is significantly elevated in late-stage, hormone-refractory tumors.[31,32] Coincidently, promoter of *PLAU* gene is extensively methylated in benign and early-stage PCa, whereas it gets demethylated in highly invasive malignant cells.[33] This is a perfect exemplary correlation showing manipulation of gene expression and consequently cancer progression by one single epigenetic mechanism. DNA demethylation predominantly occurs in the intergenic and intronic areas, particularly at repeated sequences including the heterochromatic satellite DNA and interspersed transposable elements. It is postulated that DNA hypomethylation induces genomic instability and mutation events, thus contributing to oncogenesis and cancer progression.

HISTONE MODIFICATION

Histone proteins are the most important structural components of nucleosome, the fundamental chromatin unit. They are highly conserved and alkaline, so they tightly associate with the negatively charged DNA through a series of electrostatic interactions including hydrogen bonds and salt bridges. There are several regulatory mechanisms controlling the dynamic interaction between histone and DNA, one of which is the posttranslational modifications (PTMs) of histone proteins. Various types of covalent modifications have been detected at specific amino acids on histones, including acetylation, methylation, phosphorylation, ADP-ribosylation, and ubiquitination. These enzyme-assisted modifications primarily occur at the N-terminal tails of the histones. The added moieties can affect the charge properties of the histone, thus loosening or tightening the condensed nucleosome structure. These chemical groups can also help decoy other proteins that specifically recognize the modified residues. The recruitment results in alterations of chromatin environment, so that the *cis*-regulatory elements become more closed or more accessible. Modifications of histones can have very profound influence on every DNA-associated process, such as packaging, transcription regulation, replication, recombination, and repair. For this reason, posttranslational modifications of histone proteins are also called histone codes in analogy to genetic code, as they add an extra layer of complexity to cellular phenotypes that were originally thought to be predominantly determined by DNA sequences.

Increasing evidence suggests involvement of histone modifications in the onset and progression of PCa. Distinct types of modifications, especially methylation and acetylation, show differential intensities between normal and cancerous samples. For example, one study evaluated methylation of H3K4 and H3K9 as well as pan-acetylation of H3 and H4 by immunohistochemistry in a tissue microarray containing 23 nonmalignant and 113 adenocarcinoma samples.[34] Di- and trimethylation of H3K9 and acetylation of H3 and H4 were all significantly reduced in cancer tissues. In contrast, all three methylation states of H3K4 were upregulated in androgen-independent tumors and correlated with clinical–pathological parameters. These findings suggest that changes in the overall intensity of certain histone modification may be closely associated with cancer and that they are predictive of clinical outcomes. In an independent report, levels of acetylated H3K9, H3K18, and H4K12 and dimethylated H4R3 and H4K4 were analyzed in 183 primary PCa tissues.[35] Remarkably, patterns of all these five modifications in combine stratified patients into groups showing differential risks of 10-year tumor recurrence.

Interestingly, different numbers of the same moiety on particular histone residue may display distinct immunohistochemical signals at different stages of PCa.[36] For instance, comparably strong staining of H4K20 trimethylation was observed in normal, localized as well as metastatic, hormone-naïve tumors, whereas castration-resistant tissues showed the weakest levels of mono- and dimethylation. In addition, monomethylation signal was significantly correlated with lymph node metastases, while dimethylation correlated with the Gleason score. Other histone marks that have been investigated in prostate tumorigenesis include H3K18 acetylation and H3K27 methylation. In a cohort of 279 PCa cases, Kaplan–Meier analysis showed a significant association between levels of acetylated H3K18 and increased risk of tumor relapse.[37] Intensities of H3K27 mono- and trimethylation have been reported to positively correlate with aggressive tumor features.[38,39] Strikingly, concentrations of H3K27me3 could be detected in cell-free circulating nucleosome from peripheral blood of patients by an enzyme-linked immunosorbent assay (ELISA). They were significantly lower in men with metastatic disease than in those with localized or local advanced tumors.[40] All these studies convincingly demonstrate that changes in global levels of certain histone-modifying events are associated with increased risks of PCa recurrence and poor survival. However, most of the indications were based on immunostaining assays that heavily depend on the quality of the antibodies for data interpretation, and it is still deliberative as for how individual histone codes change in PCa. Even so, cumulative evidence implies that patterns of histone modifications may distinguish cancer cells from their normal counterparts or metastatic disease from organ-confined tumors. All the studies that demonstrate such differential intensities of certain histone codes are summarized in **Table 1**. Therefore, epigenetic patterns of histone modifications, especially methylation and acetylation, may function as promising biomarkers for PCa diagnosis and prognosis.

Although being less characterized than methylation or acetylation, other types of histone modifications are also indicated in PCa development and progression. Phosphorylation of histone variant H2AX at Ser139 has been confirmed in multiple PCa cell lines, and this posttranslational modification helps recruit essential components for DNA damage repair at sites containing double-strand breaks (DSB) and activate checkpoint proteins for cell cycle arrest.[41] Monoubiquitination of H2A was noticeably lower in prostate tumors compared to the paired normal tissues,[42] while monoubiquitination of H2B at K120 has been indicated in the control of self-renewal property of PCa stem cells.[43] In general, alterations in a particular histone mark directly reflect the aberrant expressions or activities of the enzymes that orchestrate this epigenetic program. Approximately 50% of the histone methyltransferases encoded by the human genome, for instance, are now linked to diseases and in particular to cancer.[44] It is intriguing to find that a lot of these protein enzymes are involved in activation of critical signaling pathways in PCa, which will be elaborated in a latter section. This further supports an indispensible role of epigenetics in regulation of central signaling driving prostate carcinogenesis and tumor progression.

NONCODING RNAS

Noncoding RNAs (ncRNAs) are evolutionarily conserved RNA molecules that are transcribed from DNAs but not translated into proteins. Over years, there are heated debates regarding biological significance of these transcripts. ncRNAs were initially considered as

Table 1: Histone modifications that display differential intensities or patterns in prostate cancer

Histone modifications/molecules detected	*Results*	*Indications*
H3K9ac, H3K18ac, H4K12ac, H4R3me2, and H3K4me2	Levels of all these five modifications predicted tumor recurrence independently	Prognosis
	Lower levels of H3K4me2 and H3K18ac are associated with poorer prognosis	
H3K4me1/2/3, H3K9me1/2/3, H3ac, and H4ac	H3ac and H3K9me2 levels discriminate PCa and nonmalignant prostate tissue	Diagnosis, prognosis
	H3K4me1 was a significant predictor of PSA recurrence following radical prostatectomy	
	H3K4me1/2/3 levels were significantly increased in hormone-refractory prostate cancer	
H4K20me1/2/3	H4K20me3 staining was at equally strong levels in normal tissues, localized PCa, mPCa, and CRPC	Prognosis
	H4K20me2 staining was weakest in CRPC, no difference between normal and localized PCa, showed a significant correlation with the Gleason score	
	H4K20me1 staining was weakest in CRPC, significantly correlated with lymph node metastases	
	H4K20 methylation levels were not associated with PSA recurrence after radical prostatectomy	
H3K9me2, H3K4me2, and H3K18ac	Lower levels of H3K9me2 predict poorer outcome for individuals with prostate cancers	Prognosis
H3K9ac, methyl cytidine, and ISWI (SNF2H and SNF2L)	Staining of H3K9ac was decreased from BPH to LGPIN and HGPIN, with the lowest levels in prostatic adenocarcinoma	Diagnosis
H3K4me2, H3K18ac	High levels of either marker are independently associated with increased risk of relapse	Prognosis
H3K27me1/2/3	H3K27me1/3 levels were increased in mPCa and CRPC compared to localized PCa and normal prostate tissue	Prognosis
	H3K27me2 levels were lower in mPCa than in localized PCa or in CRPC	

ac: acetylation; me: methylation; PCa: prostate cancer; PSA: prostate-specific antigen; mPCa: metastatic PCa; CRPC: castration-resistant PCa; BPH: benign prostatic hyperplasia; LGPIN: low-grade prostatic intraepithelial neoplasia; HGPIN: high-grade prostatic intraepithelial neoplasia

byproducts of excessive activity of RNA polymerase II and therefore arisen from transcriptional noise.[45] Now, a plethora of research has demonstrated that individual ncRNAs are involved in a variety of fundamental biological processes, such as translation, RNA splicing, and DNA replication. Most importantly, studies find mutations, abnormal expression levels, or imbalanced supply of certain ncRNAs, which can cause human diseases including PCa.[46]

ncRNAs can be classified into small (sncRNAs) and long (lncRNAs) groups, depending on their molecular lengths. sncRNAs, in general, are <200 nucleotides. Based on their structural features and distinguishable functions, sncRNAs can further be categorized into microRNA (miRNA), small-interfering RNA (siRNA), small nuclear RNA (snRNA), and piwi-interacting RNA (piRNAs). The best-characterized type of sncRNAs in cancers is microRNAs (miRNAs), which act to block protein syntheses through either mRNA cleavage or translational inhibition.[47] A large number of studies have been carried out to determine miRNA profiles in PCa.[48,49] Both oncogenic and tumor suppressor miRNAs have been identified, so aberrations of their expression levels contribute to prostate pathogenesis and promote the malignant properties. For instance, miR-221/222 are commonly upregulated in PCa. They boost growth of both prostate carcinoma cells and xenografts by blocking expression of cyclin-dependent kinase inhibitor $p27^{kip1}$, which results in cell cycle progression at G1-to-S phase.[50] On the other hand, downregulation of miR-145 has been observed in PCa. Its decrease is related to more advanced tumor grades and higher risks of biochemical recurrence.[51] One of the targets of miR-145 is Fascin homolog 1 (*FSCN1*), an actin-binding protein that increases invasiveness of cancer cells and facilitates immune suppression.[52] This in part explains why miR-145 inhibits proliferation, migration, and invasion of PCa cells. Another miRNA molecule that has been repeatedly indicated in tumor suppression is miR-34a. In nearly 80% of primary prostate carcinomas, the promoter of *MIR34A* gene is methylated. Hence, miR-34a expression is decreased in PCa, particularly in CD44-positive cell population that possess tumor-initiating properties. CD44 is a direct target of miR-34a, so overexpression of this miRNA inhibited clonogenic capacity, halted tumor growth, and blocked metastasis of CD44+ PCa cells.[53] This study strongly suggests that miR-34a is a promising therapeutic agent against prostate cancer stem cells. Just like the abovementioned cases, multiple miRNAs show differential expression patterns in normal versus cancer cells, and their levels correlate well with pathological statuses of prostate tumors or clinical behaviors of cancer patients. In particular, miRNAs are chemically stable in fresh or even formalin-fixed tissues, and they have even been detected in many biological fluids, such as serum, urine, blood, and saliva.[54] All of these features make microRNAs highly promising as excellent biomarkers. miRNA profiling in PCa is warranted though, since outcomes of miRNA expression in clinical samples are conflicting from study to study, and no clear-cut miRNA panel has been defined.

ncRNAs that are longer than 200 nucleotides are defined as lncRNAs, and they regulate gene expression in both *cis*-acting and *trans*-acting modes. In detail, when the genomic location of lncRNA lies in close proximity to, or even within, the protein-coding target loci, transcriptional interference occurs so that elongation of lncRNA transcript suppresses initiation of adjacent transcription.[55] Alternatively, lncRNAs regulate gene expression in *cis* by direct binding to regulatory DNA elements nearby and leading to either dissociation of the preinitiation complex[56] or coating of the chromatin region.[57] lncRNAs can also control distal transcriptional events in *trans* via crosstalk with RNA polymerases, transcription factors, or epigenetic regulators. Formation of the ribonucleoprotein complex may change subcellular localization or enzymatic activities of bound proteins.[58–60] Taken together, lncRNAs-mediated transcriptional regulation is extremely context specific and renders diverse biological consequences, so both oncogenic and tumor-suppressive functions have been implied for various lncRNA molecules.

The lncRNA prostate cancer antigen (*PCA3*) is one of the first lncRNAs that are identified to be highly specific for prostate cancer.[61,62] Its level is dramatically elevated in more than 95% of prostatic tumors[63] and can be detected even in a small chunk of specimens

that contained <10% PCa cells.[64] Now, it is feasible to determine *PCA3* amount in urinary samples, and a nucleic acid sequence-based amplification assay, called uPM3, was then developed in such clinical setting. This assay uses a primer-dependent technology to continuously amplify *PCA3* RNA under isothermal conditions.[65,66] The quantitative score reliably predicted the incidence of prostate carcinogenesis when combined with other clinical information including prostate volume, PSA level, inflammation, or patient's age.[67] These findings provide the fundamental basis for clinical application of *PCA3* as a useful biomarker for PCa diagnosis. In 2012, Progensa™ PCA3 urine test was approved by the US Food and Drug Administration (FDA), which helps determine the need for repeat prostate biopsies in men who had previous negative results. In addition, *PCA3* modulates expression of multiple genes that are involved in a variety of important biological processes such as angiogenesis, epithelial–mesenchymal transition (EMT), cell adhesion, and apoptosis. Consequently, overexpression of the lncRNA *PCA3* induces cell proliferation and drives PCa progression. Therefore, *PCA3* has also been suggested as a therapeutic target for advanced PCa.

Another prostate-specific lncRNA is called second chromosome locus associated with prostate-1 (*SChLAP1*). Emerging evidence suggests that *SChLAP1* may serve as a prognostic biomarker, since *SChLAP1* expression is pronouncedly elevated in patients treated with radical prostatectomy, which independently predicts biochemical recurrence and chances of cancer-related mortality.[68] Mechanistically, *SChLAP1* interacts with SNF5, a crucial subunit of SWItch/sucrose nonfermentable (SWI/SNF) complex, disrupts the chromatin recruitment of this complex, and counteracts the tumor-suppressive effect of SWI/SNF.[69] Prostate cancer gene expression marker 1 (*PCGEM1*) is also a prostate-specific lncRNA, which is exclusively expressed in glandular epithelial cells of human prostate. Its level is induced by the male hormone androgen, increased in 56%–84% of PCa compared with matched normal specimens, and tends to associate with high-risk PCa patients.[70,71] Overexpression of *PCGEM1* promoted cell proliferation and colony-forming potential of PCa cells,[70] implying an oncogenic function of this lncRNA in PCa. Numerous other lncRNAs have been implicated in prostate carcinogenesis and tumor progression, such as focally amplified lncRNA in epithelial cancer (*FALEC*), which is induced in a hypoxic environment and functionally enhances proliferation, migration, and invasion of PCa cell;[72] PCa associated transcript 1 (*PCAT1*), which post-transcriptionally upregulates *c-MYC* by interfering with miRNA-mediated inactivation of the oncogene.[73] The numbers of lncRNAs that contribute to malignant properties of PCa cells keep growing. Besides the above-mentioned examples, we provide a relatively complete list in **Table 2**. In summary, noncoding RNAs have dynamic roles in transcriptional regulation and are involved in a variety of biological processes, all together leading to the development of pathogenesis of PCa.

Most recently, a cell-derived microvesicle, called exosome, was found to serve as a critical communicating messenger between cells. Accumulating evidence indicates that exosomes from tumor microenvironment play key roles in regulation of PCa cell survival, proliferation, metastases, angiogenesis, and immune surveillance.[74,75] Exosome is 30-100 nm in size, composed of lipid bilayer membrane, and encapsulates bioactive molecules including ncRNAs. Several work characterized the ncRNAs content in exosomes that are released by PCa cells. One report investigated the levels of 742 miRNAs in serum-derived circulating microvesicles from 78 PCa patients and 28 normal individuals.[76] Interestingly, miR-375 and miR-141 were significantly enriched in exosomes from patients with metastases compared with those without recurrent PCa. Another study found a specific group of lncRNAs abundantly present in PCa exosomes, which harbor perfect binding sites for the seed regions of highly expressed miRNAs.[77] Due to the strong association of specific exosomal RNA biomarkers with PCa, Exosome Diagnostics Inc. launched a urine-based assay called ExoDx® Prostate (IntelliScore) to measure the exosomal expression of *ERG*, *PCA3*, and *SPDEF*. The gene expression score in combination with results of standard-of-care tests represents a more accurate way of discriminating high-grade PCa from low-grade or benign disease and significantly expedites the biopsy decision-making process. Tremendous efforts are ongoing to exploit certain ncRNAs for diagnosis and prognosis of PCa, and scientists attempt to develop minimally invasive procedures in circulating tumor cells, cell-free DNAs, or extracellular vesicles to detect these epigenetic biomarkers.

CROSSTALK OF EPIGENETIC MACHINERY WITH FUNCTIONAL SIGNALING IN PROSTATE CANCER

Signaling pathway is a hierarchical network that controls cell functions by a series of molecules working together. It is a fundamental mechanism of cell growth, metabolism, division, and other molecular processes. One of the most critical signaling in PCa is the action of the steroid hormone receptor protein, androgen receptor (AR). It is now widely accepted that AR plays a pivotal role in normal development and malignant transformation of prostate epithelial cells.[78] Misregulation of AR signaling has been identified in almost every step of PCa initiation and progression, for instance, *AR* gene mutations and amplification;[79] aberrant AR activity by unbalanced interaction with its cofactors;[80] and upregulation of constitutively active AR splice variants.

Loss of AR protein expression has been seen in as many as 20%–30% of androgen-independent tumors, which is partly attributed to epigenetic silencing by promoter hypermethylation.[81,82] Although the frequency of *AR* promoter methylation in general appears to be low, this type of epigenetic regulation seems to be more prevalent in castration-resistant PCa (CRPC). It increases from 0–20% incidence in untreated primary cancer to 13%–28% in hormone-refractory tissues. It is thus highly clinical relevant to identify this AR-negative subgroup of PCa, and implication of DNA methylation in mediating downregulation of AR expression will have a profound effect on the treatment regimens for the metastatic, hormone-refractory PCa.

A lot of histone marks as well as the corresponding enzymes are intimately participated in regulating AR competency. Accumulating data from chromatin immunoprecipitation (ChIP) assays shows that acetylated histone H3 colocalizes with AR binding sites and potentiates transactivation of the androgen-responsive genes.[83] Epigenetic regulatory proteins that recognize this specially modified histone play key roles in regulation of AR-dependent transcriptional profiles. For example, the bromodomain-containing protein 4 (BRD4), which binds to acetylated lysine residues on histones through the structural module bromodomain, associates with AR, facilitates recruitment of this nuclear receptor to its target loci, and therefore contributes to the aggressive phenotypes of PCa cells.[84] Lysine-specific histone demethylase 1A (KDM1A), which specifically demethylates mono- and dimethylated H3K4 and K9, resides in a complex with AR to drive transcription of androgen-dependent genes. Higher expression of the demethylase is associated with poorer outcome in primary PCa patients.[85,86] Likewise, lysine-specific demethylase 4C (KDM4C) also forms complexes with AR to stimulate androgen-dependent growth of PCa cells, suggesting that KDM4C may contribute to prostate tumorigenesis as well.[87] Another epigenetic enzyme that has been repeatedly implied in regulation of AR signaling is the methyltransferase enhancer of zeste homolog 2 (EZH2), which is the catalytic subunit of

Table 2: Long noncoding RNAs that have been implicated in prostate cancer, their expression levels in cancer compared to normal samples, and their potential clinical associations as well as biological functions

LncRNAs	*Expression in cancer versus normal counterparts*	*Clinical relevance*	*Proposed functions in PCa*
CCAT2	↑	Prognostic/predictive biomarker	Positively associates with the histological grade and tumor stage; high CCAT2 expression levels had poorer overall survival and progression-free survival
CTBP1-AS	↑	Therapeutic target	Located in the anti-sense strand of CTBP1; promotes castration-resistant prostate tumor growth by regulating epigenetically cancer-associated genes
DANCR	↑	Therapeutic target	Promotes invasion and migration via mediating the binding of EZH2 on the TIMP2/3
DRAIC	↓	Prognostic/predictive biomarker	Prevents the migration and metastatic spread of PCa cells
FALEC	↑	Prognostic/predictive biomarker, therapeutic target	Induction of hypoxic environment; promotion of proliferation, migration, and invasion
GAS5	↓	Therapeutic target	Prevents the androgen/AR complex binding to target promoter regions; downregulated in CRPC; suppresses PCa cell progression and tumor growth by inactivating the PI3K-Akt-mTOR signaling pathway
H19	↓	Diagnostic biomarker, therapeutic target	Upregulation of H19 represses cell migration; targets TGF-β1 to repress cell migration
HCG11	↓	Prognostic/predictive biomarker	Downregulation of HCG11 is associated with a poor prognosis in PCa
HOTAIR	↑	Prognostic/predictive biomarker, therapeutic target	Binds to AR to prevent its ubiquitination and degradation; upregulated in CRPC upon deprivation therapies; promotes cell growth and invasion
LINC01296	↑	Prognostic/predictive biomarker, therapeutic target	Promotes PCa growth and metastasis through activating the PI3K-Akt-mTOR signaling pathway
LincRNA-p21	↓	Prognostic/predictive biomarker	Promotes apoptosis and suppresses PCa cell proliferation and colony formation
LncRNA-ATB	↑	Therapeutic target	Increases cell proliferation and promotes EMT
LOC283070	↑	Therapeutic target	Transition of LNCaP cells to an androgen-independent state; promotion of cell proliferation and migration
LOC400891	↑	Prognostic/predictive biomarker	Overexpressed in DU-145 and 22RV1 PCa cell lines; promotion of PCa cell proliferation and metastasis;
LOC44040	↑		Overexpressed in PC3 and 22RV1 but not in DU-145 PCa cell line; promotion of PCa cell proliferation and metastasis
MALAT-1	↑	Diagnostic/prognostic biomarker	Suppressing expression in castrated nude mice delayed tumor growth and reduced metastasis
MEG3	↓	Therapeutic target	Suppresses cell proliferation and induces apoptosis via activating p53
NEAT1	↑	Prognostic/predictive biomarker	Overexpression in CRPC and resistant to ADT or AR antagonists
PCA3	↑	Diagnostic biomarker, therapeutic target	Activation of AR signaling; promotion of cell growth; modulates angiogenesis and EMT; regulation of tumor suppressor, PRUNE2
PCAT1	↑	Prognosis, therapeutic target	Upregulation of c-Myc; increases PCa cell proliferation, migration, invasion and suppresses apoptosis
PCAT14	↓	Prognostic/predictive biomarker	Overexpression of PCAT14 suppresses the invasive capabilities of PCa cells
PCAT18	↑	Diagnostic biomarker, therapeutic target	Highly expressed in CRPC; promotion of tumor progression by AR signaling
PCAT29	↓	Prognostic/predictive biomarker, therapeutic target	First AR-repressed lncRNA that functions as a tumor suppressor. Low PCAT29 expression correlated with higher rates of biochemical recurrence
PCAT5	↑	Therapeutic target	Exclusively overexpressed in ERG-positive PCa and CRPC tissue
PCGEM1	↑	Diagnostic/prognostic biomarker, therapeutic target	Activation of AR and c-Myc; promotion of cell proliferation, migration and invasion; regulates miR-145 which is considered as a tumor suppressor
pIncRNA-1	↑	Therapeutic target	Enhances cell proliferation and reduces apoptosis; protects the AR from miRNA-mediated suppression; promotes EMT via TGF-β1 pathway
POTEF-AS1	↑	Therapeutic target	Promotes cell growth, inhibited apoptosis and repressed genes through the Toll-like receptor signaling pathway
PRNCR1	Disputed		Knockdown of PRNCR1 reduced the viability of PCa cells and the activity of the AR
PVT1	↑	Prognostic/predictive biomarker, therapeutic target	Regulates PCa cell viability and apoptosis via miR-146a
SChLAP1	↑	Prognostic/predictive biomarker	Promotion of cell invasion and metastasis; negative regulator of miR-198; counteracts the tumor-suppressive effects of SWI/SNF
SNHG1	↑	Prognostic/predictive biomarker	Promotion of PCa cell proliferation in association with miR-199a-3p
SOCS2-AS1	↑	Therapeutic target	Promotes castration-resistant and androgen-dependent cell growth
SPRY4-IT1	↑	Diagnostic biomarker	Knockdown of SPRY4-IT1 inhibits cell proliferation and invasion, and increases apoptosis
TRPM2-AS	↑	Diagnostic biomarker	Regulates the expression of oncogenes and is associated with a poor prognosis

Contd

Table 2: Contd....

LncRNAs	*Expression in cancer versus normal counterparts*	*Clinical relevance*	*Proposed functions in PCa*
UCA1	↑	Prognostic/predictive biomarker, therapeutic target	Acts as a competitive endogenous RNA; enhances tumor cell proliferation, invasion and migration; promotes EMT
ZEB1-AS1	↑	Therapeutic target	Promotes the PCa cells proliferation and migration via binding and recruiting MLL1 to the *ZEB1* promoter region

lncRNAs: long noncoding RNAs; SWI/SNF: SWItch/sucrose nonfermentable; EZH2: enhancer of zeste homolog 2; TIMP: tissue inhibitors of metalloproteinase; PCa: prostate cancer; AR: androgen receptor; CRPC: castration-resistant PCa; PI3K: phosphatidylinositol-3 kinase; TGF-β1: transforming growth factor-β1; EMT: Epithelial-mesenchymal transition; Akt: protein kinase B; CCAT2: colon cancer-associated transcript 2; CTBP1: C-terminal binding protein 1; AS: antisense RNA; DANCR: differentiation antagonizing non-protein coding RNA; DRAIC: downregulated RNA in cancer, inhibitor of cell invasion and migration; FALEC: focally amplified lncRNA on chromosome 1; GAS5: growth arrest-specific 5; ATB: activated by TGF-β; HCG11: human chorionic gonadotrophin 11; HOTAIR: HOX transcript antisense intergenic RNA; MALAT-1: metastasis associated lung adenocarcinoma transcript-1; MEG3: maternally expressed gene 3; NEAT1: nuclear-enriched autosomal transcript 1; PCA3: prostate cancer gene 3; PCAT1: prostate cancer associated ncRNA transcript 1; PCGEM1: prostate cancer gene expression marker 1; POTEF: POTE ankyrin domain family member F; PRNCR1: prostate cancer non-coding RNA 1; PVT1: plasmacytoma variant translocation 1; SChLAP1: second chromosome locus associated with prostate 1 isoform 6; SNHG1; small nucleolar RNA host gene 1; SOCS2: suppressor of cytokine signaling 2; SPRY4-IT1: SPRY4 intronic transcript 1; TRPM2: transient receptor potential melastatin 2; UCA1: urothelial carcinoma associated 1; ZEB1: zinc finger E-box binding homeobox 1; ↑: upregulated; ↓: downregulated

the polycomb repressive complex 2 (PRC2) and methylates H3K27. It was found to serve as an AR coregulator and orchestrates AR-regulated gene signatures that either block nonprostatic differentiation[88] or promote cell cycle progression.[89] Monoubiquitination of histone H2B at K120 got increased upon androgen stimulation within the transcribed regions of AR target genes, and this increment was coupled to the enhanced proliferation of PCa cells.[90]

Growing body of evidence documents an important role of noncoding RNAs in modulating AR activity. Genome-wide screening of transcriptional changes identified large numbers of noncoding transcripts being induced or suppressed upon androgen stimulation, such as miR-125b, miR-21, and PCAT29.[91–93] These molecules then carry out a series of gene expression regulations or interact with various transcription (co)factors, cascading down the androgen-AR axis. Reciprocally, a plethora of ncRNAs directly manipulates expression of AR. It is not surprising that both transcript and protein levels of AR are suppressed by several miRNAs, such as miR-145[94] and miR-205.[95] The suppression is mediated by direct binding of miRNAs to the 3'-UTR of *AR* gene and dependent on the Argonaute protein, which is the active component of the RNA-induced silencing complex (RISC) cleaving the target mRNA strand. On the other hand, the lncRNA *PlncRNA-1* sponges these AR-targeting miRNAs and masks the miRNA-response elements in *AR* transcript by serving as a competing endogenous RNA (ceRNA). As a result, *PlncRNA-1* promotes PCa growth by upregulating AR expression.[96] Interestingly, the most common splice variant of AR, AR-v7, is transcriptionally governed by ncRNAs as well. Two lncRNAs, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT-1*) and *PCGEM1*, are reported to increase expression of v7. They directly interacted with the spliceosome-associated factors SF2[97] and U2AF65,[98] respectively, and subsequently enhanced the binding capacity of these factors with *AR* pre-mRNA. Considering the strong linkage of AR-v7 to the aggressive and treatment-resistant phenotypes of PCa cells, inhibition of lncRNAs represents appealing therapeutic strategy for advanced, late-stage disease.

Not only acting to regulate AR level, both small and long ncRNAs may also influence competency of AR signaling via direct crosstalk with either the nuclear receptor itself or coregulators. One of the paradigms in such scenarios is the lncRNA HOX transcript antisense intergenic RNA (HOTAIR). This 2.2-kb long transcript was demonstrated to serve as a scaffold for both polycomb repressive complex 2 (PRC2) and lysine-specific demethylase 1 (LSD1)/REST corepressor (CoREST)/REST using 5' and 3' domains of the lncRNA, respectively.[99] This enables the assembly of both histone-modifying complexes and leads to coordinated regulation of histone marks. Considering the profound impact that EZH2 and LSD1 have on AR activity, it is plausible that HOTAIR orchestrates the epigenetic landscape flanking AR binding locations and therefore facilitates the formation of a welcoming chromatin environment to assist AR-DNA interaction. Recently, HOTAIR was found to physically interact with AR, block the docking site for the E3 ubiqutin ligase murine double minute 2 (MDM2), and thereby prevent AR from degradation. As a consequence, AR was accumulated so abundantly that it induced transactivation even in the absence of androgen.[100] In summary, a hierarchical network of ncRNAs, which can be regulated by androgen, in return can control both expression levels and transcriptional activity of AR.

In addition to androgen-AR axis, epigenetic programs play vital roles in activation of other biologically important signaling that is involved in cancer stem cell self-renewal, EMT transition, angiogenesis, *etc*., such as Wnt/β-catenin, phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt), and Hedgehog pathways.[101] Ligand productions or expressions of any auxiliary proteins along the signaling axes can be modulated by particular epigenetic mechanisms. Direct crosstalk between epigenetic machinery and the master transcription (co)factors determines the downstream cascade of biological effects. Meanwhile, epigenetic regulatory proteins are frequently targets of, or posttranslationally modified by, one of these signaling pathways. All in all, signal circuits and epigenetic network are interwoven with each other, constituting a complex and hierarchical feedback loop.

EPIGENETIC THERAPY

Epigenetic molecules contain characteristics that make them superior as powerful, noninvasive diagnostic, or prognostic indicators of PCa, either independently or when combined with other clinical parameters: they usually can be reproducibly quantified and resistant to various storage conditions because of relatively stable chemical structures; breakthroughs in technologies make it achievable to detect them using small amounts of clinical samples; they can possibly be measured in a wide range of bio-organic fluids. In addition to serving as biomarkers, epigenetics has been widely investigated for anticancer drug design purposes. This is because first, every epigenetic machinery is involved in control of multiple processes conforming to the cancer complexity, and hence manipulation of one specific epigenetic program may implement corrections for numerous cell functions that have gone awry in cancers; second, normal epigenetic patterns can be restored in theory by reversing the abnormal activities of the enzymes exercising the catalytic effects. So far, six epigenetic drugs, two DNA methyltransferase (DNMT) inhibitors and four histone deacetylase (HDAC) inhibitors, have been approved by FDA for the treatment of myelodysplastic syndrome, multiple myeloma, and T

cell lymphoma.[102–105] However, none of them are applied in clinical practice for solid tumors including PCa, as most of the epigenetic drugs show promising yet limited effects in preclinical settings. Hence, more efforts are necessary to fully understand the molecular mechanisms underlying epigenetic functions in PCa pathologies and to develop targeted epigenetic drugs as new initiatives against the disease (**Figure 2**).

DNMT inhibitors 5-azacytidine and 5-aza-2'-deoxycytidine, which are nucleoside analogs, have been evaluated *in vivo* in PCa xenografts and displayed some curative effects.[106] Moreover, 5-aza-2'-deoxycytidine was demonstrated to suppress hormone-independent growth of PCa in castrated mice.[107] Furthermore, RG108 and disulfiram, two nonnucleoside compounds that supposedly inhibit enzymatic activity of human DNMTs, exerted antitumor effects in PCa cell lines and animal models.[108,109] Both types of DNMT inhibitors are thought to reactivate tumor suppressor genes, such as *GSTP1*, *APC*, *RASSF1A*, and *RAR2*, by specific demethylation of their promoters.[110] Unfortunately, in spite of all these promising results in preclinical models, there are only a few clinical trials testing DNMT inhibitors in PCa patients with either modest activities or severe side effects.[111,112]

A panel of small molecule inhibitors of the enzymes that regulate histone codes are currently under intensive evaluation to assess their anticancer potentials. Several EZH2 inhibitors that selectively block its methyltransferase activity have been developed and they all demonstrated dose-dependent inhibition of H3K27me3 without triggering EZH2 protein degradation.[113–115] Some of them are currently under clinical trials in patients with hematologic malignancies. Because both H3K27me3-dependent and H3K27me3-independent mechanisms were indicated in mediating the antitumor effects of EZH2, the mechanism of drug action in PCa cells needs further investigation.[89,116] Bromodomain-containing proteins, especially the bromodomain and extra-terminal motif (BET) family members, represent another resourceful repertoire of epigenetic drug targets. BET inhibitors, such as JQ1, I-BET151, and I-BET762, destroy interaction between BET domain and modified residues, affect a large variety of biological processes, and demonstrate promising outcomes in early clinical trials.[117] In terms of PCa, JQ1 exhibited a drastic antineoplastic activity particularly in castration-resistant cells by abolishing BRD4-mediated chromatin recruitment of AR and transactivation.[84] OTX015, a derivative of JQ1, is a novel orally bioavailable inhibitor of BRD2, BRD3, and BRD4. It exhibited significant synergism, together with AR antagonists, in inhibiting the growth of metastatic, hormone-refractory cells.[118] Intriguingly, administration of OTX015 potently diminished PCa stem cell population in sphere-forming assays, providing a compelling strategy for the treatment of the most aggressive PCa cells that seed the bulky disease.[119] Another group of epigenetic drug precursors that have been extensively studied is the inhibitors of histone demethylase LSD1. Several highly selective LSD1 inhibitors have been identified recently, such as NCL-1, HCI-2509, and namoline.[120–122] All of these lead compounds are potent, reversible, and selective in terms of impairing H3K4-demethylating activity. They suppressed the androgen-independent growth of CRPC cells both *in vitro* and *in vivo* with no apparent adverse effects.[122,123] Pan-demethylase inhibitors have also been designed and synthesized. Several of them caused growth arrest and substantial apoptosis in cancer cells including prostate, but had little effects on nonmalignant cells.[124] Finally, two clinical trials are currently being conducted with phenelzine sulfate, a potent inhibitor of monoamine oxidase (MAO) that is closely homologous to LSD1, and therefore the prototype drug is considered as a nonspecific inhibitor of the demethylase. In these trials, the lead agent was administered either alone to treat patients with relapsed PCa that has not metastasized (NCT02217709) or in combination with docetaxel for progressive PCa cases after first-line therapy with docetaxel (NCT01253642). **Table 3** lists all the ongoing or terminated clinical trials testing epigenetic drugs that target either DNA methylation or certain histone-modifying enzymes in PCa.

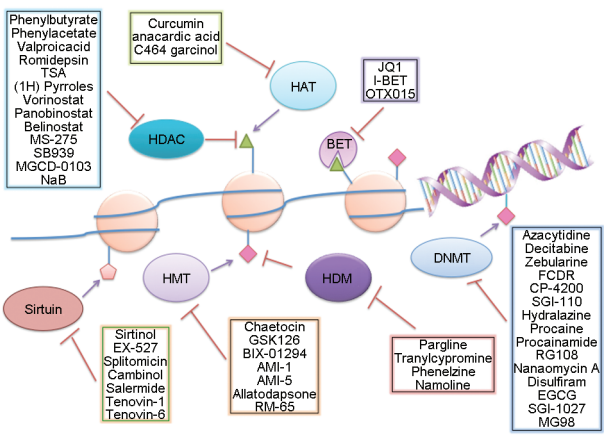

Figure 2: DNA/histone-modifying enzymes are targeted for the development of epigenetic drugs. HAT: histone acetyltransferase; HDAC: histone deacetylase; HMT: histone methyltransferase; HDM: histone demethylase; DNMT: DNA methyltransferase; BET: the bromodomain and extra-terminal domain. Green triangle: acetyl functional group; pink diamond: methyl modification; orange pentagon: ADP-ribose moiety; FCDR: 5-fluorodeoxycytidine; SGI-110: guadecitabine; RG108: N-phthalyl-l-tryptophan; EGCG: epigallocatechin-3-gallate; MG98: DNA methyltransferase-1 by antisense.

As for ncRNAs, a phase I clinical trail (NCT01829971) was launched in April 2013 by Mirna Therapeutics (Austin, TX, USA) to evaluate the anticancer potential of a liposome-formulated miR-34a mimic (MRX34). This was the first attempt to use a miRNA as an innovative therapy for cancer, and a lot of hindrances still exist regarding the clinical application of ncRNAs, such as the optimal way of delivery, the potential side effects, and therapeutic regimen management. There has been a recent explosion of interest in utilizing exosome as a delivery vector of therapeutic molecules, such as miRNAs, to target PCa. For example, exosomes that were released from adipose-derived stromal cells carried and unloaded miR-145 into the cocultured PCa cells. The cargo then inhibited PCa cell proliferation and induced apoptosis, possibly via downregulation of anti-apoptotic protein Bcl-xL.[125] This proof-of-principle study implied an encouraging clinical application of exosomes as a pharmaceutical formulation for epigenetic drugs.

Even though most of the drugs targeting certain epigenetic marks are still in preclinical or early phase trial stages, it is appealing to screen for novel compounds that achieve robust inhibition of aberrant epigenetic codes for cancer therapy.

CONCLUSION

It is now generally accepted that epigenetics contributes to the development of nearly every stage of PCa. Targeting certain epigenetic mechanism may represent an alternative approach to current prevalent treatment. More work is warranted to gain deeper understanding of the roles of epigenetic modifications in control of cancer-specific gene expression, the crosstalk among various epigenetic marks, and the close cooperation with critical signaling pathways. Increased insights into these epigenetic regulatory mechanisms will definitely foster successful

Table 3: Clinical Trails testing epigenetic drugs in prostate cancer

Drug name	*Target*	*Combination*	*Phase*	*Indication*	*Identifier/registry number*	*Status*	*Results*
Azacitidine	DNMT	Phenylbutyrate	II	PCa	NCT00006019	Completed	No study results or publications provided
Azacitidine	DNMT		II	PCa	NCT00384839	Completed	No study results or publications provided
Azacitidine	DNMT	Docetaxel and prednisone	I/II	mCRPC with postchemotherapy	NCT00503984	Terminated	Significant demethylation of GADD45A was observed with azacitidine treatment
Vorinostat	HDAC		II	Progressive metastatic prostate cancer	NCT00330161	Completed	No PSA decline ≥50%; median time to progression and overall survival were 2.8 and 11.7 months, respectively
Vorinostat	HDAC	Docetaxel	I	Advanced solid tumor including prostate cancer	NCT00565227	Terminated	Closed due to toxicity; no study results or publications provided
Vorinostat	HDAC		I	Advanced solid tumor including prostate cancer	NCT00005634	Completed	No study results or publications provided
Vorinostat	HDAC	Doxorubicin	I	Metastatic or locally advanced solid yumors	NCT00331955	Completed	No study results or publications provided
Vorinostat	HDAC	mTOR inhibitor temsirolimus	I	Metastatic prostate cancer	NCT01174199	Terminated	No value in finding efficacy; no study results or publications provided
Panobinostat	HDAC		II	mCRPC	NCT00667862	Completed	11.4% of the patients were alive without progression of disease at 24 weeks; 14.3% of the patients demonstrated a decrease in PSA, but none ≥50%
Panobinostat	HDAC	Docetaxel and prednisone	I	CRPC	NCT00493766	Terminated	Because of a strategic decision by Novartis; no study results or publications provided
Panobinostat	HDAC	Bicalutamide	I/II	CRPC	NCT00878436	Completed	It was registered a >50% PSA decline by 9 months of therapy
Romidepsin	HDAC		I	Solid tumors with liver dysfunction	NCT01638533	Recruiting	No study results or publications provided
Romidepsin	HDAC		II	mCRPC	NCT00106418	Completed	There was no significant cardiac toxicity; two patients achieved a confirmed radiological partial response lasting ≥6 months, along with a confirmed PSA decline of ≥50%
SB939	HDAC		II	CRPC	NCT01075308	Completed	PSA response in 6% patients; CTC response in 64% patients
Valproic acid	HDAC		II	Progressive, nonmetastatic prostate cancer	NCT00670046	Recruiting	No study results or publications provided
Sulforaphane	HDAC		II	Recurrent prostate cancer	NCT01228084	Completed	5% of the patients who achieve a 50% decline in PSA levels
MGCD-0103	HDAC	Docetaxel	I	Advanced cancer tumors including prostate cancer	NCT00511576	Terminated	Celgene terminated its collaboration agreement with MethylGene for the development of MGCD0103; no study results or publications provided
Curcumin	HAT		II	PCa	NCT02064673	Recruiting	No study results or publications provided
Curcumin	HAT	Taxotere	II	mCRPC	NCT02095717	Active, not recruiting	No study results or publications provided
Phenelzine	HDM		II	Nonmetastatic recurrent prostate cancer	NCT02217709	Recruiting	No study results or publications provided
Phenelzine	HDM	Docetaxel	II	PCa patients with progressive disease after first-line therapy with docetaxel	NCT01253642	Terminated	Low enrollment No study results or publications provided
OTX015	BET		I	CRPC	NCT02698176	Terminated	No study results or publications provided
OTX015	BET		I	CRPC	NCT02259114	Completed	No study results or publications provided

BET: bromodomain and extra-terminal; HDM: histone demethylase; HAT: histone acetyltransferase; HDAC: histone deacetylase; DNMT: DNA methyltransferase; PCa: prostate cancer; CRPC: castration-resistant PCa; mCRPC: metastatic CRPC; PSA: prostate-specific antigen; CTC: circulating tumor cell

clinical applications of epigenetic codes as biomarkers of cancer risk stratification, to predict therapy response, or to provide alternative treatment options for PCa.

Epigenetic therapy is still in its infancy and there is a long way to go for the goal of personalized medicine. There are several factors that hinder the initiatives of applying epigenetic drugs in clinical practice.

First, the biological functions of majority of the above-discussed epigenetic enzymes have not been fully elucidated or validated. Considering the highly heterogeneous nature of PCa, it is quite likely that effect of a particular epigenetic pattern on growth of cancer cells varies from case to case and context specific. Second, besides the problem of identifying druggable targets, another challenge is to explore the mechanisms of action and the pharmacological behavior of any epigenetic drugs. Lack of success in clinical trials that test tool compounds targeting epigenetic programs in PCa raises the concerns about their potencies, specificities, and side effects. This can possibly be due to misses of molecular readouts that authentically reflect the effectiveness of the drug or biomarkers that can be used to properly stratify patients. Despite of all these difficulties, with breakthroughs in technologies precisely mapping particular epigenetic marks, with appearance of new paradigms of medicinal chemistry, and with more comprehensive knowledge of epigenetic functions, the future of epigenetic therapy is bright.

COMPETING INTERESTS

Both authors declared no competing interests.

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