Epigenetic Modulations and Lineage Plasticity in Advanced Prostate Cancer

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ABSTRACT

Prostate cancer is the most common cancer and second leading cause of cancer death in American men. Anti-androgen therapies are part of the standard of therapeutic regimen for advanced or metastatic prostate cancers. However, patients who receive these treatments are more likely to develop castration-resistant prostate cancer (CRPC) or neuroendocrine prostate cancer (NEPC). In the development of CRPC or NEPC, numerous genetic signaling pathways have been under preclinical investigations and in clinical trials. Accumulated evidence shows that DNA methylation, chromatin integrity and accessibility for transcriptional regulation still play key roles in prostate cancer initiation and progression. Better understanding of how epigenetic change regulates the progression of prostate cancer and the interaction between epigenetic and genetic modulators driving NEPC may help develop a better risk stratification and more effective treatment regimens for prostate cancer patients.

Keywords: Prostate, castration-resistant prostate cancer (CRPC), neuroendocrine prostate cancer (NEPC), epigenetics, molecular genetics, lineage plasticity

Word counts:130

INTRODUCTION

Prostate cancer is a frequently detected cancer in the United States for men over 50 years of age. Despite recent therapeutic advances, prostate cancer still represents a major urological disease associated with substantial morbidity and mortality [1, 2]. Androgen deprivation therapy (ADT) has been the cornerstone treatment for advanced and metastatic prostate cancers. Despite initial high response rates, cancer management with ADT is of limited duration; nearly all men eventually develop progressive prostate cancer following ADT. This is referred to as castration-resistant prostate cancer (CRPC) [3]. This progression may be caused by a series of mechanisms, which include but not limit to AR-dependent mechanisms of resistance [4, 5] and a glucocorticoid receptor-adaptive resistance mechanism [6]. CRPC is also conferred by a frequently occurring AR splice variant. One of the most common variants is AR variant 7 (ARv7) [7-8].

In light of the accumulated evidence for developing resistance mechanisms, epigenetic alterations result in enhanced or attenuated transcriptional activity and play important roles in pro-oncogenic role of AR signaling. This is mediated by either DNA/chromatin methylation, histone modification to affect the direct binding of AR to DNA, posttranslational modifications in the AR itself or AR-cofactors leading to gain-of-function [9].

Another increasingly accepted mechanism of resistance is lineage plasticity, which appears in multiple cancer types, including prostate cancers [10-12]. Lineage plasticity denotes a process by which cancer cells change from one morphological and functional cell type to another, under the influence of environmental pressures. In the context of prostate cancer treatment, lineage plasticity refers to a shift in cellular phenotype from an AR-dependent adenocarcinoma to an AR-indifferent neuroendocrine carcinoma, which likely occurs following ADT. Lineage

plasticity has a markedly different epigenetic profile, low AR signaling, and acquired expression of neuroendocrine stem cell markers [13]. Given that the appearance of neuroendocrine cells in prostate cancer is associated with aggressive tumors and poor prognosis [14], better understanding of the genetic and epigenetic mechanisms by which NEPC develops is necessary to design therapeutic strategies for NEPC patients.

Epigenetic changes drive the progression of prostate cancer

Epigenetic controls of transcriptional regulation include DNA methylation, histone modification, and chromatin remodeling. These epigenetic modifications drive carcinogenesis in prostate cancers [15]. A genomic landscape study of primary and metastatic prostate cancers has identified mutations in many epigenetic regulators and chromatin remodelers in up to 20% of advanced prostate cancers [11, 15-18]. These mutations are associated with high-grade prostate cancer [19] and development of CRPC and NEPC [20].

DNA methylation

A family of DNA methyltransferases (DNMT) catalyzes the transfer of a methyl group from S- adenosylmethionine to the fifth carbon of cytosine residue to form 5-methylcytosine (5mC) [21]. DNMT1, one of the major subtypes of DNMT, has tumor suppressor activity in early-stage prostate cancers but oncogenic activity in advanced prostate cancers, and metastasis through induction of epithelial–mesenchymal transition and cancer stem cell phenotype [22, 23]. Aberrant DNA methylation is usually associated with aggressive clinicopathological features and poor survival. Interestingly, our recent study suggests that DNMT1 regulates expression of steroid 5-α reductase 2 (SRD5A2). SRD5A2 gene methylation in promoter region is associated

with better survival for CRPC patients treated with ADT. This is the first study to our best knowledge that showing increased methylation level is associated with better prognosis in advanced prostate cancers (data in preparation). Further exploring the epigenetics of DNMT and SRD5A2 may help us better understand the relationship between epigenetics and development of advanced prostate cancers and guide us in choosing the best therapeutic strategies for management.

DNA demethylation

Although DNA methylation has been known for decades, how DNA demethylation occurs remained unclear until the discovery of TET enzymes and the ability to oxidize 5mC to 5hmC [24]. TET family proteins promote locus-specific reversal of DNA methylation in normal cells which ensures that DNA methylation patterns and gene expression are precisely regulated [25]. TET-mediated 5hmc level in genomic DNA is usually decreased in many types of solid tumors, including prostate cancers [26, 27]. In prostate cancers, the tumor-suppressive TET family proteins, which are involved in DNA demethylation, are repressed; in particular, TET2 is involved in the AR signaling and has an important role in prostate cancer development. For example, Takayama et al [28] reported that the AR-induced miR-29 family specifically downregulates TET2 in prostate cancers, resulting in activation of AR and mTOR signaling and reduced expression of both TET2 and 5hmC which are associated with prostate cancer progression. In a similar study, TET proteins were found to suppress tumor progression and invasion partly through downregulation of critical gene methylation [29]. Storebjerg et al [30] reported that TET family proteins and the epigenetic mark 5hmC have been found to be a novel

candidate prognostic biomarker. Further studies are warranted to explore the epigenetic mechanisms of TET proteins in CRPC and NEPC.

Histone modifications

The role of histone modifications has been recognized in prostate cancer for a long time. For instance, WHSC1 (also known as NSD2), the histone dimethyl methyltransferase was found to be stabilized by AKT and its stabilization plays a causal role in driving prostate cancer metastasis [29]. Aytes et al [31] demonstrated that NSD2 is a robust marker of lethal metastatic prostate cancer and a potential biomarker for prostate cancer diagnosis. Study also found that another histone demethylase JMJD1A functions as a key coactivator for AR by epigenetic regulation of H3K9 methylation. JMJD1A recruits HNRNPF to promote alternative splicing of AR-V7 in prostate cancer cells, and silencing of JMJD1A reduced the level of AR-V7 in prostate cancer cells [32]. JMJD2A, another subtype of histone demethylase has also been found to drive prostate carcinogenesis through transcription factor ETV1 [33]. In addition, histone acetyltransferase Bromodomain-containing proteins, particularly BRD4 has been found to bind to acetylated lysine on histones and play an important role in prostate cancer progression [34].

A recent study further reported a novel chromatin-regulated mechanism of AR transcriptional regulation being mediated by the tyrosine kinase ACK1 (also known as TNK2) [35]. ACK1/TNK2 phosphorylates histone H4 at Try88 upstream of the AR transcription start site, leading to WDR5/MLL2 complex-mediated increase of AR transcription and the epigenetic regulatory circuits drive CRPC progression. Reversal of the pTry88-histone H4 epigenetic marks by the ACK1 inhibitor (R)-9bMS sensitizes naive and ADT-resistant prostate cancer cells and reduces AR expression and halts CRPC growth [35].

LSD1 (also known as KDM1A), a FAD-dependent demethylase, functions as a transcriptional repressor of AR-regulated enhancers through H3K4 and H3K9 demethylation and as an AR coactivator through interaction with RCOR1/CoREST and phosphorylation of histone H3 Thr-6 (H3T6ph) [36]. LSD1 was found to promote CRPC also through epigenetic programming to induce CENPE, a centromere binding protein and mitotic kinesin, expression [37]. LSD1 is significantly upregulated in CRPC, which may suggest serve as a predictive biomarker of aggressive prostate cancer. Targeting LSD1 in conjunction with AR antagonists may also be a promising therapeutic approach to treat CRPC [38]. LSD1 inhibitors are under clinical investigation for prostate cancer and other cancers [39].

Repressor Element 1-Silencing Transcription factor (REST)

REST is an important epigenetic regulator which is often dysregulated in NEPC [40]. REST is widely expressed in embryonic stem cells, where it orchestrates a set of epigenetic modifications, thus mediating the plasticity of gene chromatin by recruiting co-repressors, such as EZH2 and LSD1, to repress neuronal differentiation. The linkage between epigenetic mechanisms and neurogenesis has been documented by REST [41]. In prostate cancers, REST mediates AR repression and is a key mediator of neuroendocrine transdifferentiation mediated by androgen depletion [42]. REST reduction plays an important role in hypoxia-mediated neuroendocrine differentiation of prostate cancers through activation of AMPK/mTOR pathway [43].

Polycomb group proteins

The polycomb group proteins are other epigenetic modulators with strong links to cellular dedifferentiation and malignant progression. Polycomb group proteins consist of two main repressive complexes, PRC1 and PRC2. PRC2 binds to H3K27me3 and represses neighboring nucleosomes. The methylation requires physical interaction between EZH2 and EED, the two core catalytic subunits of PRC2 [44, 45]. In the context of prostate cancers, EZH2 overexpression is associated with cancer progression [46] and can be inhibited by microRNA-101 [47]. Clermont et al [48] established the in vivo model of ADT-induced NEPC using patient-derived xenografts and also showed that EZH2 and CBX2, a PRC2 component, are the most highly expressed epigenetic regulators in the NEPC tumor model compared with the original prostate adenocarcinoma (PADC). Other studies also demonstrated that upregulation of EZH2 is a well-established driver of NEPC progression [11, 17, 49, 50].In the MYCN-driven NEPC mouse model, EZH2 functions directly with MYCN to suppress the AR signaling which promotes NEPC development [51]. Although the precise role of EZH2 in the emergence and maintenance of the NEPC phenotype remains unclear, EZH2 has been identified as an important target with promising therapeutic effects [51]. In addition, BMI1, the component of PRC1, was reported to be upregulated in prostate cancers. BMI1 ubiquitylates histone H2A through its catalytic subunits RING1A or RING1B [52] and plays an important role in epigenetic regulation of cell differentiation and cancer stem cell self-renewal [53].

In one word, aberrant epigenetic modifications are crucial for prostate cancer progression. Recent whole-genome bisulfite sequencing coupled with transcriptome data revealed that multiple genetic pathways are also epigenetically dysregulated in NEPC including those involved in cell–cell adhesion, development of epithelial–mesenchymal transition, and the

regulation and maintenance of stem cells [11]. Investigating the interaction between epigenetic and genetic regulators might be an innovating research field for the advanced prostate cancer.

Lineage plasticity driving development of NEPC

Clinical and genomic profiling suggest that NEPC may originate *de novo* from a scattered neuroendocrine cells (usually less than 1% of total cell population) in PADC [54]. CXCL8 (also known as IL-8), chemokine receptor CXCR2, and CXCL8–CXCR2–TP53 signaling keep the neuroendocrine cells in adenocarcinoma in a quiescent state. A TP53 mutation in the neuroendocrine cells can destroy the balance and lead to rapid progression of NEPC from PADC [55]. However, in the majority of cases, NEPC-like cells may develop from a population of luminal-epithelial cells following ADT [56]. Recent developed next-generation sequencing (NGS) has made it possible to sequence prostate cancer of different stages, from PADC to NEPC [10, 57, 58], and identify different genetic mechanisms underlying lineage plasticity or PADC-to-NEPC transdifferentiation. For example, NGS verified that NEPC not only has mutation or loss of TP53, but is also enriched for loss of RB, loss of AR and AR target gene expression, and overexpression of MYCN (chromosome 2p24, encoding N-MYC) and AURKA (encoding Aurora-A) [11, 17, 59]. Other studies also suggested that neuroendocrine transdifferentiation of PADCs are driven by variable genetic mechanisms such as overexpression of SRRM4 [60], hypoxia-mediated signaling [43, 61], PKC-lambda/iota reduction [62], and elevated HP1α expression [63]. In addition, tumor-associated macrophages (TAM) are an important component of tumor microenvironment. Recently, Zarif et al [64] showed that TAM can reprogram tumor phenotype and play a major role in the emergence of a neuroendocrine phenotype in prostate

cancers. Targeting these signals in the prostate cancer microenvironment could potentially augment immunotherapeutic approaches in NEPC.

Crosstalk between genetic and epigenetic modulators in NEPC progression

Loss of RB1 and TP53

Cooperative loss of RB1 and TP53 was present in 53.3% of NEPC vs 13.7% of PADC [11]. One study also reported that combined loss of RB1 and TP53 in the anti-androgen–sensitive LNCaP-AR prostate cancer cell line facilitated acquisition of ADT resistance and led to increased lineage plasticity. However, this resistance and phenotypic shifting to NEPC could be reversed by re-expressing RB1 and TP53 proteins [49]. Ku et al [65] reported a similar finding as RB1 loss facilitated phenotypical shifting from luminal cells to neuroendocrine-like cells and metastasis of PADC initiated by PTEN mutation. The additional loss of TP53 in this model accelerated the onset of expression of neuroendocrine markers and anti-androgen resistance. In addition, they also demonstrated that RB1 and TP53 loss in prostate cancers upregulated epigenetic-important reprogramming factors EZH2 and SOX2; thus creating a stem cell–like epigenetic environment permissive for lineage plasticity. The repression of EZH2 or SOX2 by epigenetic modulation (such as histone H3K27 methylation inhibitor) reversed or delayed the lineage switch and restored sensitivity to ADT [49, 65]. SOX2 is one of four transcriptional factors (OCT4, SOX2, LIN28, and NANOG) involved in regulating self-renewal and reprogramming fibroblasts to induced pluripotent stem cells [66]. These factors were reported to be sufficient to reprogram somatic cells into induced pluripotent stem cells, promoting lineage plasticity in prostate cancer following ADT [49]. Therefore, loss of TP53 and RB1-mediated

lineage plasticity is closely cooperative with epigenetic mechanisms in driving NEPC development.

MYCN and AURKA amplification

Concurrent MYCN (encoding N-MYC) and AURKA (encoding Aurora-A) gene amplification are more significant events in NEPC, compared with PADC [17, 51]. Forty percent of NEPC overexpressed both genes, compared with 5% of PADCs. N-MYC is critical for maintenance of human embryonic stem cells as well as neural stem and precursor cells [67, 68]. It is well demonstrated that N-MYC drives NEPC from human prostate epithelium [17, 51]. Lee et al [22] also reported that N-MYC and activated AKT1 are sufficient to transform human prostate epithelial cells to PADCs and NEPCs. Although direct pharmacological inhibition of N-MYC has proven challenging, N-MYC can be stabilized by N-MYC/Aurora-A dimerization. This complex is targetable by Aurora-A inhibitors [22, 69, 70]. Aurora-A inhibitors, such as alisertib [71] and CD532 [70], have demonstrated significant anti-tumor activity associated with a significant reduction in MYCN protein levels [51]. A clinical trial also reported that some advanced prostate cancer patients with Aurora-A and N-MYC activation achieve significant clinical benefit from alisertib [72].

Moreover, Berger et al [73] uncovered a potential mechanism by which overexpressed N-MYC interacts with AR cofactors (such as FOXA1 and HOXB13). These cofactors may alter MYCN DNA binding through competition at N-MYC binding sites or by altering the chromatin accessibility. The authors also described that epigenetic bivalent markers (H3K4me3/H3K27me3) and EZH2 activity specifically associated with lineage-defining genes

were reprogrammed by N-MYC. Finally, they demonstrated that N-MYC–induced gene expression and epigenetic changes may accurately classify the patient cohort.

Overexpression of SRRM4

SRRM4 is a master regulator required for neural differentiation from embryonic stem cells [74]. Some SRRM4-targeted genes, such as REST and PHF21A, in neural cells have been identified in NEPC, suggesting that SRRM4 is functionally active in NEPC and might drive neuroendocrine transdifferentiation of PADCs [60]. Consistent with these findings, data obtained from clinical prostate cancer cohorts confirmed that SRRM4 is highly expressed in 50% of NEPCs versus 3% of PADCs [75]. Li and colleagues [60] performed whole transcriptome analyses on patient samples [17, 40] and determined that SRRM4 is an upstream regulator of REST, an epigenetic regulator, and regulates REST splicing in prostate cancers. They also demonstrated that SRRM4 is a powerful driver of NEPC transdifferentiation [60]. In other studies, SRRM4 stimulated adenocarcinoma cells to express NEPC biomarkers, which was exacerbated by ADT [76]. SRRM4-mediated development of NEPC under ADT was further augmented with the loss of TP53 and RB1 [60]. SRRM4 may also induce the expression of key reprogramming factors, such as SOX2, to promote lineage plasticity, thus driving NECP development [77]. However, the mechanisms of induction of SRRM4 remain unclear. No NEPC-specific mutations were found in the SRRM4 promoter; therefore, induction of SRRM4 gene expression could be controlled by epigenetic mechanisms. One study identified that several SRRM4 target genes are histone acetyltransferases or de-methyltransferases (e.g., MEAF6 and BHC80) and may promote NEPC progression through epigenetic mechanism [78]. Therefore,

crosstalk between epigenetics and gene expression of SRRM4 warrants further investigations. Nevertheless, these findings may guide the development of novel therapeutics aimed at NEPC.

FOXA1 loss and FOXA2 gain

The FOXA family proteins, particularly FOXA1 and FOXA2, are implicated in the development of NEPC. Studies showed that FOXA1 is transiently upregulated in localized prostate cancer but downregulated in CRPC [79, 80] and further downregulated in NEPC [81]. FOXA1 loss upregulates CXCL8 in NEPC [82] and CXCL8-activated MAPK/ERK phosphorylation promote neuroendocrine differentiation of prostate cancer cells. Moreover, FOXA1 has been also reported to play pioneering and reprogramming roles in lineage conversion [83]. Genome-wide location analyses show that FOXA1 physically interacts with TET1 proteins, a 5-methylcytosine dioxygenase. TET1 can potentiate FOXA1 recruitment through regulating local DNA demethylation and concomitant histone H3 lysine 4 methylation and FOXA1 binding can be reduced following TET1 depletion [84]. This study unraveled the potential crosstalk mechanism between FOXA1 signaling and epigenetics and further explained the molecular mechanisms of how FOXA1 drives prostate cancer progression through epigenetic modifications. On the other hand, overexpression of FOXA2 was reported in a genetically engineered mouse model of NEPC [85] and the N-MYC/AKT1–driven NEPC [22]. Assessment of FOXA2 expression in clinical prostate cancer specimens interestingly showed that FOXA2 expression is not detectable in PADCs, whereas it is highly expressed in NEPCs [86]. HIF1α is a hypoxia-induced gene and interacts with REST[43] and also partners with FOXA2 to promote prostate cancer development[87]. Therefore, whether FOXA2 could induce phenotypical switching from PADC to NEPC via the HIF1A-REST–mediated epigenetic mechanism requires

further investigation. Nevertheless, given the important regulatory roles of FOXA1/FOXA2 in NEPC progression, and their close relationship with epigenetic mediators, a better understanding of crosstalk between FOXA family proteins and epigenetic modulators in NEPC could discover a possible new therapeutic strategy.

Hypoxia mediated neuroendocrine differentiation

Hypoxia is an evident in prostate cancer development, increasing with clinical stage, patient age [88]. Recently, Guo et al [61] reported that hypoxia promotes neuroendocrine transdifferentiation in PADCs through ONECUT2 signaling. ONECUT2 is found to regulate hypoxia-induced gene expression in NEPCs and activate SMAD3, a cofactor of HIF1A. In another study, it was reported that ONECUT2 also suppresses the translational activity of AR and represses the expression of FOXA1 through regulating PEG10, thus driving NEPC. The authors also showed that treatment with a small molecule inhibitor, CSRM617, against ONECUT2 has promising effects in prostate cancers [89]. Kim et al [90] reported that PEG10 is highly expressed in NEPC. Targeting PEG10 reduces proliferation rate and expression of neuroendocrine markers. It has been known for years that PEG10 is a paternally expressed imprinted gene. Similar to other imprinted genes, PEG10 expression is closely regulated by epigenetics [91]. Moreover, in the context of advanced prostate cancers, whether hypoxia drives NEPC development through PEG10 mediated epigenetics remains unknown. In other studies, Lee et al [92] reported that histone demethylase KDM3A served as a transcriptional coactivator of HIF1A in regulation of their target genes in prostate cancer. In a similar study, KDM3A was reported to be positively regulated by PHF8, indirectly sustaining H3K4me3 levels on select hypoxia-inducible genes in prostate cancers [93]. As mentioned above, Lin et al also reported

that REST is involved in hypoxia-mediated neuroendocrine differentiation of prostate cancers [43]. Altogether, these studies indicate that epigenetic signatures may be one of important mechanisms involved in hypoxia-induced NEPC progression.

PKC-lambda/iota reduction

In a recent study, Reina-Campos et al [62] analyzed clinical metastatic NEPC samples and identified that PKC-lambda/iota is reduced in NEPC during ADT, which lead to mTORC1/ATF4-driven upregulation of serine synthesis. Serine is the major source of one-carbon units for methylation reactions that occur via the generation of S-adenosylmethionine [94]. In this study, serine is found to facilitate tumor growth and to epigenetically switch to the NEPC phenotype. Interestingly, when the PKC-lambda/iota deficiency cells were treated with decitabine or cycloleucine, which competitively inhibits the enzyme methionine adenosyltransferase resulting in the inhibition of S-adenosylmethionine synthesis reducing methyl donor supplies for the DNMT reaction, both treatments significantly reduced the expression of basal and NEPC biomarkers and had potent antiproliferative effects on PKC-lambda/iota deficiency cells. Because serine is a nonessential amino acid, treatments targeted at blocking serine synthesis will only impact prostate cancer, without toxic side effects to benign tissues [62].

Elevated* HP1α *expression

Ci et al [63] recently identified 36 heterochromatin-related genes by analysis of patient-derived xenograft, multiple patient cohorts, and genetically engineered mouse models. They showed that NEPC have a distinctive heterochromatin gene signature compared with PADCs.

Within this gene signature, elevated HP1α expression is an early event in NEPC development and is associated with a poor prognosis. They also showed that HP1α promotes neuroendocrine transdifferentiation of PADCs following ADT. Silencing of HP1α reactivates AR and REST expression and inhibits NEPC tumor growth [63]. The chromodomain of the HP1 proteins recognizes H3K9me3 and this interaction forms the basis for further recruiting of other heterochromatin-associated proteins, thereby initiating chromatin modification and gene silencing [95]. Interestingly, DEK1, an epigenetic regulator, was reported to interact directly with HP1α and significantly enhance its binding to trimethylated H3K9 (H3K9me3). The DEK1-mediated epigenetic event has been identified as an important role in driving NEPC [96]. Altogether, these studies suggest that an HP1α-dependent mechanism is a potential functional driver promoting NEPC development via regulating specific epigenetic mechanism.

Silencing of RASAL3 and glutamine secretion in tumor microenvironment

It has been long known that cancer-associate fibroblasts can promote the progression and/or initiation of prostate epithelial cells via regulating extracellular matrix and growth factors [97]. Mishra et al [98] also reported that epigenetic changes in prostatic cancer–associated fibroblasts initiate stromal–epithelial interactions. This interaction facilitated tumor growth and development of resistance to ADT. Analysis of DNA methylome revealed that epigenetic silencing of RASAL3, a Ras inhibitor, in prostatic cancer–associate fibroblasts activated Ras signaling, thus driving macropinocytosis-mediated glutamine synthesis. Glutamine uptake by prostate cancer epithelia promotes tumor neuroendocrine differentiation indicating the Ras-mediated metabolic reprogramming in prostatic cancer–associate fibroblasts driving NEPC. The RASAL3 epigenetic silencing and glutamine secretion by prostatic fibroblasts can be further

enhanced following ADT. The authors also suggested that therapeutic strategies to prevent glutamine uptake be considered in conjunction with ADT.

Altogether, lineage plasticity has been an important mechanism driving neuroendocrine transdifferentiation in PADCs. Different genetic signaling pathways and tumor microenvironments could be involved in the plasticity mechanism. However, many of the components in driving lineage plasticity have been found to have a bridge with epigenetic modulators (**Figure 1**). Therefore, a better understanding of how the interplay between epigenetics and plasticity mechanisms helps us to gain insights into the advanced prostate cancer progression and develop effective therapeutic approaches for advanced prostate cancer patients.

Potential therapeutic options for NEPC

Targeting epigenetic effectors will have clinical benefits for prostate cancer patients. BET bromodomain inhibitors, histone methyltransferase inhibitors, DNMT inhibitors, HDAC inhibitors, and many other numerous epigenetics therapies (**Figure 2**) are currently in clinical trials. Active and completed clinical trials involving epigenetic drugs are summarized in **Table 1**.

Not only have drugs targeting epigenetic modulators been developed, but other regimen-targeting genetic modulators are under preclinical and clinical investigations (**Table 2**). For example, alisertib/MLN8237 inhibits the interaction between N-MYC and its stabilizing factor Aurora-A, thus inhibiting N-MYC signaling and delaying NEPC growth [72]. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate in NEPC is also under clinical trial. A single therapeutic agent is often not enough for treatment of advanced prostate cancers. Therefore, combined therapeutic agents, such as olaparib combined with cabazitaxel, carboplatin, and

prednisone, and Radium-223 combined with dexamethasone in aggressive prostate cancers, are also in clinical trials. Antabuse (disulfiram) administration of intravenous copper chloride in combination with oral disulfiram in CRPC and NEPC are also under clinical investigation. This therapeutic combination acts by activating reactive oxygen species (ROS) and inhibiting DNMT and ubiquitin/proteasomal signaling [99, 100]. Platinum-based therapy in combination with docetaxel is also a common treatment option for NEPC [101-102].

However, development of epigenetic therapy is still challenging. One of major obstacles is that most epigenetic therapies have nonspecific distributions and exert their functions over an extensive transcriptional network and are not able to achieve cancer cell specificity, leading to undesirable off-target and toxic side effects [103-105]. As our understanding of the epigenetic alterations in human prostate cancers becomes more advanced, the field will have many opportunities to translate this into impactful clinical management of prostate cancers, especially CRPC and NEPC. Among the different classes of epigenetic alterations in prostate cancer, DNA methylation alterations are the best characterized, and are, therefore, the most mature for translation to biomarkers for prostate cancer screening/diagnosis, risk stratification, and treatment/disease monitoring.

CONCLUSION

Prostate cancer progression, neuroendocrine transdifferentiation, and treatment responses are all regulated by epigenetics. A genomic landscape study has identified aberrant epigenetic events in CRPC and NEPC development. Although many genetic modulators involving the development and maintenance of advanced prostate cancers have been under preclinical and clinical investigation, recent data reveal that many of the signaling pathways are also

epigenetically dysregulated in advanced prostate cancers including those involved in cell to cell adhesion, development of epithelial–mesenchymal transition, and the regulation and maintenance of stem cells. Therefore, epigenetic targeting may represent an alternative therapeutic regimen to treatment with genetic modulator inhibitors. More studies are warranted to gain better understanding of the role of epigenetics in the development of advanced prostate cancers and crosstalk with lineage plasticity of NEPC progression. Moreover, advanced prostate cancers have complex molecular expression and epigenetic patterns, leading to inconsistent results when using a single class of drugs. Ongoing preclinical and clinical trials have shed light on the advantage of combination therapies and on improving treatment for this deadly disease.

Highlights

- Patients who receive anti-androgen treatments are more likely to develop castration resistant prostate cancer.
- A genomic landscape study has identified aberrant epigenetic events in CRPC and NEPC development.
- Epigenetic targeting may represent an alternative therapeutic regimen for advanced prostate cancer.
- Ongoing preclinical and clinical trials have shed light on the advantage of combination therapies

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Figure Legends

Figure 1. This model depicts the molecular and phenotypic events associated with progression to castration-resistant prostate cancer (CRPC) and neuroendocrine prostate cancer (NEPC) and indicates both genomic and epigenetic modulators involving the lineage plasticity of prostate cancer. Genomic modulators include cooperative loss of RB1 and TP53; N-MYC and AURKA amplification; SRRM4 overexpression; FOXA1 loss and FOXA2 gain; Hypoxia-mediated signaling; elevated HP1α expression as well as PKC-lambda/iota reduction and serine synthesis. Epigenetic/reprogramming modulators include EZH2, EED, polycomb group proteins, SOX2, REST, and DEK1. ADT: Androgen deprivation therapy.

Figure 2. Graphic summary of epigenetic regulation in prostate cancer and potential therapeutic options. Chromatin structure and accessibility to transcriptional factors are regulated dynamically by epigenetic mechanisms. Writers such as DNA methyltransferase (DNMT), histone lysine acetyltransferase (HAT), and histone lysine methyltransferase (KMT) catalyze the addition of epigenetic marks onto either DNA or histone tails. Readers such as BRD4 recognize a specific epigenetic mark. Erasers such as histone deacetylases (HDAC) and histone lysine demethylases (KDM) remove epigenetic marks. EZH2, EED, and SUZ12 are components of PRC2. The targets shown here are either approved or under clinical trials (ME: Methyl; AC: Acetyl; DUBs: Deubiquitylating enzymes; POL II: RNA polymerase II)

TABLE 1: Epigenetic modulators used in clinical trials (either completed or active) to treat prostate cancer

Clinical Trials Identifier: (Recruitment Status)	**Intervention/Treatment**	**Targets**	**Condition/Disease**
NCT02711956 (Active)	Drug: ZEN003694 Drug: Enzalutamide	BET	Metastatic castration-resistant prostate cancer
NCT02607228 (Active, not recruiting)	Drug: GS-5829 Drug: Enzalutamide	BET	Metastatic castrate-resistant prostate cancer
NCT02259114 (Completed) NCT01987362 (Completed)	Drug: MK-8628	BET	Castration-resistant prostate cancer
NCT02705469 (Completed)	Drug: ZEN003694	BET	Metastatic castration-resistant prostate cancer
NCT02391480 (Completed)	Drug: ABBV-075 Drug: Venetoclax	BET	Prostate cancer
NCT03213665 (Recruiting) NCT01897571 (Active, not recruiting) NCT02875548 (Recruiting) NCT03217253 (Active, not recruiting)	Drug: Tazemetostat	EZH2	Advanced solid tumors including prostate cancer Metastatic malignant solid neoplasm
NCT02900651 (Recruiting)	Drug: MAK683	EED	Relapsed/refractory or recurrent/metastatic prostate cancer
NCT02712905 (Recruiting)	Drug: INCB059872 Drug: All-trans retinoic acid (ATRA) Drug: Azacytidine Drug: nivolumab	LSD1	Advanced tumor including prostate cancer
NCT02998567 (Recruiting)	Drug: Guadecitabine Drug: Pembrolizumab	DNMT	Castration-resistant prostatic cancer
NCT01118741 (Completed)	Drug: Disulfiram	DNMT	Prostate cancer
NCT00384839 (Completed)	Drug: Azacitidine	DNMT	Prostate cancer
NCT01075308 (Completed)	Drug: HDAC inhibitor SB939	HDAC	Recurrent or metastatic castration-resistant prostate cancer

NCT00670553 (Completed) NCT00667862 (Completed)	Drug: Panobinostat	HDAC	Prostate cancer Metastatic hormone refractory prostate cancer
NCT00878436 (Completed)	Drug: Panobinostat Drug: Bicalutamide	HDAC	Recurrent prostate cancer after castration
NCT00663832 (Completed)	Drug: LBH589 (i.v. panobinostat)	HDAC	Hormone refractory prostate cancer (combined with Docetaxel and Prednisone)
NCT00330161 (Completed) NCT00589472 (Completed)	Drug: Vorinostat	HDAC	Advanced prostate cancer that has progressed on one prior chemotherapy Localized prostate cancer (combined with Bicalutamide)
NCT00020579 (Completed)	Drug: Entinostat	HDAC	Advanced tumor including prostate cancer
NCT00413075 (Completed)	Drug: Oral belinostat	HDAC	Advanced tumor including prostate cancer
NCT00413322 (Completed)	Drug: Belinostat Drug: 5-Fluorouracil (5-FU)	HDAC	Advanced tumor including prostate cancer (combined with 5-FU)

Table 2: Completed or active clinical trials to treat neuroendocrine prostate cancer

Clinical Trials Identifier: (Recruitment Status)	**Intervention/Treatment**	**Targets**
NCT01799278 (Completed)	Drug: MLN8237	AURKA
NCT02963051 (Active, not recruiting)	Drug: Copper Drug: Disulfiram Drug: Copper gluconate	NPL4
NCT03179410 (Recruiting)	Drug: Avelumab	PD-L1
NCT03910660 (Recruiting)	Drug: Talabostat Mesylate plus Pembrolizumab	FAP
NCT03511664 (Active, not recruiting)	Drug: 177Lu-PSMA-617	FOLH1 (also known as PSMA)
NCT02893917 (Active, not recruiting)	Drug: Cediranib Drug: Olaparib	PARP
NCT01549951 (Completed)	Drug: Orteronel+Prednisone	CYP17 lyase
NCT02445976 (Completed)	Drug: Seviteronel	CYP17 lyase and AR inhibitor
NCT00878436 (Completed)	Drug: Panobinostat Drug: Bicalutamide	HDAC
NCT03263650 (Recruiting)	Drug: Cabazitaxel Drug: Carboplatin Drug: Prednisone Drug: Olaparib	DNA recombination