Tzu Chi Medical Journal 2021; 33(3): 224-232

Review Article

Current advances of targeting epigenetic modifications in neuroendocrine prostate cancer

*Wen-Chi Cheng[a], Hung-Jung Wang[b,c]**

[a]SDGs Teaching and Research Headquarters, Tzu Chi University, Hualien, Taiwan, [b]Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, [c]Doctoral Degree Program in Translational Medicine, Tzu Chi University and Academia Sinica, Hualien, Taiwan

Submission : 17-Aug-2020
Revision : 05-Sep-2020
Acceptance : 06-Oct-2020
Web Publication : 24-Feb-2021

Abstract

Neuroendocrine prostate cancer (NEPC) is the most lethal malignancy of prostate cancer (PCa). Treatment with next-generation androgen receptor (AR) pathway inhibitors (ARPIs) has successfully extended patients' lifespan. However, with the emergence of drug resistance, PCa tumors increasingly adapt to potent ARPI therapies by transitioning to alternative cellular lineage. Such therapy-induced drug resistance is largely driven from the cellular plasticity of PCa cells to alter their phenotypes of AR independence for cell growth and survival. Some of the resistant PCa cells undergo cellular reprogramming to form neuroendocrine phenotypes. Recent evidences suggest that this cellular reprogramming or the lineage plasticity is driven by dysregulation of the epigenome and transcriptional networks. Aberrant DNA methylation and altered expression of epigenetic modifiers, such as enhancer of zeste-homolog 2, transcription factors, histone demethylases, are hallmarks of NEPC. In this review, we discuss the nature of the epigenetic and transcriptional landscapes of PCa cells which lose their AR independence and transition to the neuroendocrine lineage. We also discuss how oncogenic signaling and metabolic reprogramming fuel epigenetic and transcriptional alterations. In addition, the current state of epigenetic therapies for NEPC is addressed.

Keywords: *Androgen receptor, Enhancer of zeste-homolog 2, Epigenetic, Neuroendocrine prostate cancer*

Introduction

Prostate cancer (PCa) is the most common cancer in males and the second leading cause of cancer-related lethality worldwide [1]. PCa as diagnosed in the first place is often an androgen-driven disease which depends on the androgen receptor (AR)-mediated signaling for tumor growth [2]. Accordingly, treatments for the AR-sensitive PCa by androgen deprivation therapy (ADT) effectively reduce tumor growth [3]. Although nearly all PCa patients respond well to ADT, some PCa cells can eventually evade ADT and restore AR signaling even in the absence of androgen, reaching a state referred to as castration-resistant PCa (CRPC) and, consequently, about 20%–25% of patients will develop to the state of metastatic CRPC (mCRPC) [4]. These tumors are still relying on AR signaling for survival, therefore treatment with next-generation AR pathway inhibitors (ARPIs) such as abiraterone and enzalutamide has significantly improved patient's survival [5,6]. Mechanistically, abiraterone and enzalutamide can blunt AR signaling, respectively, by blocking *de novo* androgen biosynthesis and competitively binding to the AR. Accumulating evidences demonstrate that prolonged treatment of CRPC or mCRPC patients with ARPIs develops therapeutic resistance. Typically, therapy resistance of the antiandrogens is mainly due to re-activation of AR by different mechanisms including genomic mutation, gene amplification, or rearrangement of the AR gene [7]. In a small proportion of patients, however, drug resistance is emerging and accompanied by a loss of AR function and its downstream signaling, leading to histological alterations that associate with the formation of epithelial–mesenchymal transition (EMT) or small-cell neuroendocrine carcinoma characteristics [8-10], which refers to neuroendocrine PCa (NEPC). NEPC has been characterized by the presence of a combination of neuroendocrine biomarkers and genomic and epigenetic features [Table 1].

In this review, we discuss the key concepts of how epigenetic and transcriptional dysregulation as a driver mechanism of cellular plasticity reprograms PCa cells to the neuroendocrine lineage (NEPC formation). We also highlight oncogenic signaling and metabolic changes which drive

Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_220_20

**Address for correspondence:*
Prof. Hung-Jung Wang,
Institute of Medical Sciences, Tzu Chi University, 701, Section 3, Zhongyang Road, Hualien, Taiwan.
E-mail: hjwang@mail.tcu.edu.tw

How to cite this article: Cheng WC, Wang HJ. Current advances of targeting epigenetic modifications in neuroendocrine prostate cancer. Tzu Chi Med J 2021;33:224-32.

Table 1: List of proposed biomarkers associated with neuroendocrine prostate cancer

Gene symbol	Gene name	Regulation*	Reference
CHGA/CHGB	Chromogranin A and B	+	[11]
NSE/ENO2	Neuron-specific enolase	+	
SYP	Synaptophysin synaptic vesicle protein p38	+	[11]
AURKA	Aurora kinase A	+	[11,12]
N-MYC	Neuroblastoma-derived Myc	+	[13]
EZH2	Enhancer of Zeste 2 (polycomb repressive complex 2 subunit)	+	[12,14]
CALC1	Calcitonin	+	[15]
FOXA2	Forkhead box A2	+	[16,17]
SRRM4	Serine/arginine repetitive matrix 4	+	[18]
POU3F2/BRN2	POU class 3 homeobox 2	+	[19]
SOX2 and SOX11	Sex-determining region Y-box 2 and 11	+	[19-21]
TMPRSS2-ERG	TMPRSS2-ERG gene rearrangement	Gene fusion	[22,23]
PEG10	Paternally expressed10	+	[24]
SCG2/SCG3	Secretogranin II and III	+	[25]
ASH1/ASCL1	Human achaete-scute homolog 1	+	[26]
NCAM1/CD56	Neural cell adhesion molecule	+	[8]
DNMT	DNA methyltransferase	+	[27]
KDMs	Histone demethylases	+	[28]
ONECUT2	Transcription factor, driver of NEPC	+	[29]
AR	Androgen receptor	-	[11]
PSA/KLK3	Prostate-specific antigen/kallikrein-3	-	[11]
RB1	Retinoblastoma tumor suppressor gene	Gene loss	[8]
TP53	Tumor protein p53	Gene loss	[30]
REST	RE1 silencing transcription factor	-	[31]
PTEN	Phosphatase and tensin homolog	-	[13,32]
CCND1	Cyclin D1	-	[33]
FOXA1	Forkhead box A1	-	[34]

*Regulation: +, upregulated; -, downregulated. NE: Neuroendocrine, NEPC: Neuroendocrine prostate cancer

epigenetic changes related to NEPC. In addition, the current progresses of clinical trials of epigenetic drugs for advanced PCa are included.

Mechanisms of therapy resistance and neuroendocrine trans-differentiation

The underlying mechanisms of CRPC are largely caused by the genetic alterations of AR, including gene amplification of the AR locus and mutation of AR, resulting in the conversion of anti-androgens to agonists. In addition, the overexpression of an AR splice variant AR-V7, an alternative splicing variant lacking the *C*-terminal androgen-binding domain, results in an androgen-independent activation of AR signaling [9,35]. Apart from deregulating AR signaling in CRPC and mCRPC, additional resistant mechanisms giving rise to the use of next-generation ADI are emerging as a potential approach to target drug resistance. For instance, the overexpression of glucocorticoid receptor (GR) confers the enzalutamide-resistant PCa by bypassing AR signaling through the mechanism, of which GR substitutes for the AR-binding sites to activate similar transcriptional programs [36,37], supporting an adaptive mechanism of AR resistance in response to ARPI treatments [11,38,39].

Another mechanism of therapy resistance is associated with cellular lineage plasticity [40]. Increasing evidences have suggested that therapy resistance has been uncovered through which the PCa cells can evade AR pathway blockade by lineage switching mechanism [41]. According to this mechanism, PCa cells acquire a specific cell lineage whose growth and survival are no longer controlled by the anti-AR drug target. An indication of the lineage switching (also referred to lineage plasticity) contributes to the anti-AR drug resistance followed by treatment of mCRPC with ARPIs and recurrence of the tumor cells exhibiting small-cell NEPC, a specific lineage that exhibits AR indifferent and resistant to current AR-targeted therapies.

Linage plasticity and neuroendocrine trans-differentiation in prostate cancer

Clinically, NEPC is an extremely aggressive cancer type of PCa, which appears resistant to any current therapies for this advanced PCa. NEPC also displays a high cell proliferation and tumor metastasis activity. Different from CRPC, which prefers to produce bone metastases, NEPC typically tends to metastasize to visceral organs such as liver and lung [42]. Histologically, NEPC displays small-cell phenotypes with high nuclear-to-cytoplasmic ratio and a high proliferative rate (indicated by Ki-67 staining). Immunohistochemical staining further indicates that NEPC specifically expresses neuroendocrine lineage markers, including synaptophysin, chromogranin A, CD56, and neuron-specific enolase, as well as the loss of the expression of AR and AR-responsive genes such as prostate-specific antigen [8,43,44]. Genetically, NEPC is also associated with genomic alterations in tumor suppressor genes

including *RB1* loss and mutation, *TP53* deletion, or *PTEN* inactivation [8,13,20,30,32]. Genome-wide sequencing studies have shown that the overall spectrum of somatic mutations between prostate adenocarcinoma and NEPC are surprisingly similar, [8,45], suggesting that additional mechanisms are required to coordinate with genomic alterations to reprogram cell fate to the neuroendocrine lineage. Not surprisingly, epigenomic abnormalities are involved in the neuroendocrine differentiation. Indeed, combining whole-genome bisulfite sequencing, a technology used for methylome analysis, and the transcriptomic analysis revealed that distinct cellular pathways are epigenetically dysregulated in NEPC, including cell adhesion, neuronal development, EMT, and the regulation and maintenance of stemness properties [46]. Taken together, numerous evidences suggest that neuroendocrine differentiation of PCa involves multiple cellular processes that are mediated by the transcription and epigenetic factors. In view of tumor microenvironment, different kinds of stroma cells in tumor itself or its surroundings, as well as other factors, also play vital roles in the induction of neuroendocrine trans-differentiation. In the following sections, we will briefly discuss the epigenetic abnormalities, regulation and maintenance of stemness [46], and EMT that have been shown to drive NEPC progression.

Epithelial-Mesenchymal Transition and neuroendocrine prostate cancer

It has been shown that neuroendocrine lineage plasticity and EMT [46] share a similar cellular mechanism, therefore it is not surprising that EMT-associated transcription factors have been implicated in NEPC. For instance, ZEB1 [47], Snail [48], Slug [49], and FOXC2 [50] promote neuroendocrine differentiation, highlighting the convergent transcriptional networks involved in NEPC and EMT. The implications of convergence between the pathways involved in EMT and NEPC suggest that therapies used for targeting EMT could also inhibit NEPC progression [51]. For example, monoclonal antibodies for targeting notch (e.g., rovalpituzumab and tarextumab) and stemness (disulfiram, an inhibitor of ALDH, which is overexpressed in cancer stem cells) are promising strategies for both EMT and NEPC.

The epigenetic basis of neuroendocrine prostate cancer

Currently available data supported that NEPC can emerge from CRPC or mCRPC following AR inhibitor treatments [8,52]. By closely examining the patient tumors during the course of disease progression using genome-wide sequencing studies, the overall spectrum of somatic mutations between prostate adenocarcinoma (PC or CRPC) and NEPC was found to be surprisingly similar [8,45]. Apart from the loss of *RB1* and *TP53* is known to contribute to progression of NEPC, the activation of pluripotency transcription factor SOX2 and the epigenetic modifier (Enhancer of Zeste-Homolog 2 [EZH2]), a histone methyl transferase subunit of polycomb repressive complex 2 (PRC2), is involved in NEPC progression [45]. In addition, protein chromobox 2 (CBX2), a PRC1 component, acts as an epigenetic modulator involved in neuroendocrine differentiation, the detailed mechanism of which is described below.

Enhancer of Zeste-Homolog 2 as a master regulator of neuroendocrine prostate cancer reprogramming

EZH2 is frequently overexpressed in PCa patients who have progressed to NEPC [8,11]. EZH2 is the catalytic subunit of the PRC2, which mediates transcriptional silencing by depositing repressive histone marker, namely tri-methylation of histone H3 at lysine 27 (H3K27 me3) [53], to suppress specific gene expressions including lineage-switching factors and to promote stemness characteristics [54]. Recently, in PCa patients, elevated activity of EZH2 (H3K27 me3 expression) has been reported in the majority of NEPC (87% NEPC vs. 46% adenocarcinoma) [8,55]. Functionally, EZH2 cooperates with lineage-guiding transcription factors to epigenetically regulate gene expression and coordinate lineage trans-differentiation [13]. In the setting of NEPC, EZH2 directly associates with N-Myc to transcriptionally repress genes that induce AR signaling-dependent CRPC [13]. Accordingly, conditional expression of N-Myc in prostate epithelial cells is sufficient to induce neuroendocrine differentiation [13,56], suggesting that N-Myc is an oncogenic driver of NEPC [13]. Consistently, N-Myc-overexpressed neuroblastomas are strongly dependent on EZH2 for cell growth and survival [57], indicating that a regulatory interplay between N-Myc and EZH2 drives the activation of neuronal differentiation. In the case of PCa, recent studies identified that N-Myc binds to the promoters of neuronal lineage-associated genes, where both repressive H3K27 me3 and active H3K4 me3 histone marks (bivalent chromatin state) were occupied. EZH2 was required to maintain the bivalency chromatin state in the N-Myc-bound genes. Furthermore, knockdown of EZH2 led to disassociation of N-Myc from the neuronal-associated genes in the NEPC organoid model [58].

EZH2 has been reported to synergize with other epigenetic modifiers to promote chromatin remodeling, facilitating lineage plasticity. For instance, EZH2 is linked to DNA methyltransferase (DNMT) activity via a scaffolding mechanism mediated by the long ncRNA HOTAIR [59]. Similarly, EZH2 complexes with a H3K36 me2 methyltransferase, nuclear receptor-binding SET domain protein 2 (NSD2). NSD2 is overexpressed in NEPC [60] and functions to reprogram epigenome by reprogramming the binding distribution of EZH2 [61]. NSD2 activity is also regulated by EZH2, which is required for the EZH2-mediated epigenetic reprogramming of PCa [62].

Recent studies are beginning to shed light on how EZH2-mediated epigenetic reprogramming facilitates neuroendocrine differentiation. In particular, EZH2 can be activated by transcription factor 4 (TCF4), a key transcription factor in Wnt/β-catenin signaling, leading to the recruitment of H3K27 me3 histone marks on the miR-708 promoter [63]. Silencing of miR-708 induces the expression of neuronatin [64], a key mediator for neuronal differentiation and stem cell-like factor CD44 [65]. Significantly, elevated

Wnt/β-catenin signaling is a feature of NEPC [66], and inhibiting TCF4 prevented NEPC trans-differentiation following androgen deprivation [63]. Moreover, EZH2 activity has been shown to be associated with cAMP-response element-binding protein (CREB) activation in PCa. Inhibition of EZH2 shows the efficacy of blocking CREB-induced H3K27 me3 and neuroendocrine differentiation [14]. EZH2 has also been reported to regulate the transcriptional activity of STAT3 [67], the nuclear factor-κB (NF-κB) pathway [68], the MAPK/ERK pathway [69,70], and the SWI/SNF chromatin remodeling complexes [71]. Further studies are required to address the complexities between the canonical PRC2-bound and noncanonical functions of EZH2 during NEPC progression.

Chromobox protein 2, a polycomb repressive complex 1 component, acts as a bridge between polycomb repressive complex 2 and polycomb repressive complex 2 to mediate neuroendocrine prostate cancer progression

Another critical PRC1 component that has been implicated a coordinate regulatory role in NEPC progression is the chromodomain protein CBX2. Mechanistically, CBX2 directly associates with the histone suppressive marker H3K27 me3 though its chromodomain, and consequently represses the transcription of target genes. This indicates that CBX2 mediates chromatin condensation in an EZH2-independent manner [72]. Functionally, CBX2 can act as a linker between PRC2 and PRC1 [73], with a concomitant upregulation in NEPC. In the NEPC tumors and xenograft tumors of NEPC, CBX2 and EZH2 are consistently overexpressed [55], confirmed by a recent transcriptomic study by comparing clinical samples of NEPC and CRPC. Therefore, it appears that aberrant PRC1 and PRC2 activities are key features of NEPC, in which upregulation of CBX2 and EZH2 correlates with the downregulation of PRC target genes [55]. Interestingly, in the lung cancer model, overexpression of both CBX2 and EZH2 appears to be significantly associated with small-cell lung carcinomas (SCLCs) rather than that of non-SCLCs, suggesting that dysregulation of these epigenetic regulators plays a major driver for neuroendocrine differentiation [55].

Repressor element 1-silencing transcription factor

In NEPC, repressor element (RE) 1-silencing transcription factor (REST) is a critical epigenetic regulator which is dysregulated in disease progression. REST functions as a transcription silencing factor and widely expressed in embryonic and pluripotent stem cells, and specifically in neuronal progenitors to modulate neuronal differentiation [74]. Mechanically, REST recruits several epigenetic co-repressors such as EZH2 (above mentioned) and lysine histone demethylase 1A (LSD1) to the RE site of neuronal genes to coordinately repress neuronal differentiation [31,75]. Clinical evidences have supported that REST downregulation is commonly observed in PCa tissues. In PCa cell lines, knockdown of REST *in vitro* can attenuate AR signaling and elevate the expression of neuroendocrine markers, indicating a specific role of REST that suppresses neuroendocrine trans-differentiation [31]. Controversially, overexpression of REST in some NEPC patients has also been observed [31]. This paradox can be explained by the overexpression of SRRM4 (serine/arginine repetitive matrix protein 4), which promotes the formation of truncated REST lacking the transcriptional repressor domain [76]. Clinically, data from PCa tissues supported that SRRM4 is specifically overexpressed in neuroendocrine tumors, say, SRRM4 was overexpressed in 50% of NEPCs versus 3% of adenocarcinomas [11,13,75].

Histone demethylases

Histone methylation had been considered to be irreversible and a nonregulated event. Until the discovery of KDM1, the first histone demethylase, which removes the trimethyl mark from H3K27, was found to be a co-activator of AR [77]. Subsequently, Jumonji C (JmjC) domain-containing molecules were found to carry lysine demethylase activity [78] Currently, there are 28 different JmjC domain-containing proteins that have been identified in human genome, of which 15 have been demonstrated to demethylate lysine residues in the H3 tail and one to demethylate the methylated arginine [79-81]. They are grouped into eight subfamilies (KDMs 1–8), with KDM8 being the newest and discovered in 2010 [82]. The most striking feature of lysine demethylases (KDM) is their exquisite specificity toward different lysine residues and different methylated forms [83,84]. Nearly all KDM family members (e.g., KDM1, KDM2A-C, KDM3A, C, KDM4A-D, KDM5A-C, KDM6B, C, and KDM8) have been found to be overexpressed in PCa, and several of these KDMs are correlated with a worse prognosis of the disease, suggesting an important regulatory role in PCa tumorigenesis by histone demethylation [83]. Currently, the well-characterized histone demethylases are KDM1 and KDM4 subfamilies. It has been shown that KDM1, KDM4A, B, and C can physically associate with AR and serve as AR co-activators [85,86]. Significantly, overexpression of KDM1s as well as KDM4A predicts the poor prognosis of PCa [80,87]. Thus, various evidences have suggested that histone demethylases have a close relationship with AR and are directly relevant to castration resistance of PCa. NEPC tumors exhibit elevated expression of the histone lysine demethylase KDM8, which functions to reprogram metabolism toward aerobic glycolysis [88]. The AR can directly recruit histone modifiers to remodel chromatin architecture and alter gene expression. LSD1 is an important regulator of AR transcriptional activity, facilitating the suppression of AR target genes via H3K4 demethylation [69]. Interestingly, LSD1-AR-mediated transactivation is associated with loss of RB1 expression, which is an important consequence for NEPC [89]. In addition, LSD1 + 8a, an LSD1 alternative splicing variant, has been shown to promote neuronal gene expression. Mechanically, the aforementioned splicing factor SRRM4 promotes the expression of LSD1+8a splicing variant and involves NEPC progression [90].

Targeting epigenetic regulators in neuroendocrine prostate cancer

Based on the progression of NEPC transcriptome and epigenome, targeting the epigenetic mechanism to reverse or delay neuroendocrine trans-differentiation is beginning to become promising. High EZH2 levels in NEPC and the association between the tumors and cellular plasticity are known to provide a rationale for developing epigenetic targeting strategies. Recent preclinical and clinical studies support this notion, as summarized in [Table 2].

EZH2 is the most well-documented dysregulated epigenetic factor in NEPC [8,55]. In preclinical NEPC models, EZH2 inhibitors have been shown to attenuate neuroendocrine phenotypes and re-sensitize ARPI treatments [13,30,58]. For example, the EZH2 inhibitor PF-06821497 is currently being tested in a Phase I study in patients with advanced/mCRPC (NCT03460977). Similarly, the Phase Ib/II trial is assessing the utility of combining the EZH2 inhibitor, CPI-1205, with enzalutamide or abiraterone in patients with mCRPC (NCT03480646).

Aurora A inhibitors such as alisertib (MLN8237), which result in destabilization of N-Myc, have also shown some efficacy in clinical trials. A Phase II clinical trial of MLN 8237 (alisertib) in NEPC patients showed a modest clinical benefit (NCT01799278). A second Phase I/II clinical trial of MLN 8237 in combination with abiraterone in CRPC with neuroendocrine differentiation was terminated

Table 2: Clinical trials for epigenetic therapies in prostate cancer

Mechanism	Clinical trial ID	Agent	Phase	Indication	Clinical status
EZH2 inhibition	NCT03009344	Tazemetostat	I	Relapsed or refractory B-cell non-Hodgkin's lymphoma	Active, not recruiting
	NCT02875548	Tazemetostat	II	Diffuse large B-cell Lymphoma; advanced solid tumors	Completed
	NCT04179864	Tazemetostat + abiraterone/prednisone tazemetostat + enzalutamide	I	mCRPC	Recruiting
	NCT03460977	PF-06821497	I	Relapsed/refractory SCLC, CRPC, and follicular lymphoma	Recruiting
	NCT02395601	CPI-1205	I	B-Cell lymphomas	Completed
	NCT03480646	CPI-1205 + enzalutamide	Ib/II	mCRPC	Active, not recruiting
	NCT01848067	CPI-1205 + abiraterone/prednisone Alisertib (MLN8237) + abiraterone + prednisone	I/II	mCRPC	Completed
	NCT03525795	CPI-1205 + Ipilimumab	I/II	Advanced solid tumors	Active, not recruiting
N-Myc inhibition	NCT01799278	MLN8237/alisertib	II	CRPC/NEPC	Completed; no appearance of new lesions for >1 month
BET inhibition	NCT02705469	ZEN003694	I	mCRPC	Completed; dose confirmation
	NCT04145375	I: ZEN003694 II: ZEN003694 + enzalutamide	I/II	mCRPC	Enrolling by invitation
	NCT02711956	I: ZEN003694 II: Enzalutamide	Ib/IIa	mCRPC	Active, not recruiting
	NCT04471974	Pembrolizumab (day 1) ZEN-3694 + enzalutamide (days 1- 21)	II	mCRPC	Not yet recruiting (2020/8 start)
	NCT02607228	GS-5829 + enzalutamide	Ib/II	mCRPC with ARPI	Completed
	NCT02698176	MK-8628	I	CRPC	Terminated
LSD1 inhibition	NCT02712905	INCB059872	I/II	Advanced malignancies; NEPC	Terminated
	NCT02217709	Phenelzine	II	Nonmetastatic recurrent prostate cancer	Active, not recruiting
	NCT01253642	Phenelzine + docetaxel	II	PCa with progressive disease	Terminated
DNMT inhibition	NCT00384839	Azacitidine for injectable suspension	II	PCa to hormonal therapy	Completed; PSA doubling time ≥3 months
	NCT03572387	5-AZA + ATRA	Pilot study	PCa with PSA-only recurrence	Recruiting
	NCT00503984	Azacitidine + docetaxel + prednisone	I/II	mPC	Terminated
	NCT03709550	Enzalutamide + decitabine	Ib/II	mCRPC	Not yet recruiting (2020/8 start)
	NCT02998567	Guadecitabine + pembrolizumab	I	CRPC solid tumors	Active, not recruiting

5-AZA: 5-azacitidine, ATRA: All-trans retinoic acid, CRPC: Castration-resistant prostate cancer, mCRPC: Metastatic CRPC, DNMT: DNA methyltransferase, PCa: Prostate cancer, ARPI: Androgen receptor pathway inhibitor, BET: Bromodomain and extra-terminal motif, NE: Neuroendocrine, NEPC: NE prostate cancer, SCLC: Small-cell lung carcinoma

early due to the severe cell toxicity and lack of clinical benefits (NCT01848067).

An epigenetic drug targeting BRD4, the BET family of chromatin readers and transcriptional regulators, is under clinical studies. Experimental data suggested that targeting BRD4 disrupts AR recruitment to its chromatin binding sites and reduces AR-dependent cell growth [91]. Treatment of BRD4 inhibitors in monotherapy or in combination with APRIs in PCa exhibits anti-tumor activities [92-94]. A Phase I/II trial is ongoing to evaluate the efficacy of the BET inhibitor ZEN003694 in combination with enzalutamide in CRPC (NCT02711956). Immunotherapy with checkpoint inhibitors, such as anti-PD-1 and anti-PD-Ll, has shown disappointing efficacy in PCa treatments [95]. In melanoma and ovarian cancer models, however, inhibition of EZH2 synergizes with immune checkpoint inhibitors to enhance the infiltration of T-cells (CD8+) to the tumor microenvironment and improve tumor killing [96]. This implicates, in PCa, that EZH2 inhibition may turn the immunologically cold prostate tumor hot. Clinical trials of EZH2 inhibitors in combination with Ipilimumab, a monoclonal antibody that activates the immune system by targeting CTLA-4, in patients with advanced solid tumors, are ongoing (NCT03525795).

Another epigenetic drug that targets LSD1 presents a promising efficacy in NEPC because LSD1 specifically overexpressed in androgen-independent PCa modulates FOXA1-dependent AR-associated reprogramming and activates stem cell-associated gene expressions [97,98]. Clinical trial of the LSD1 inhibitor INCB059872 is terminated due to the strategic business decision (NCT02712905). Another clinical trial of LSD1 inhibitor phenelzine is terminated because of low enrollment (NCT02217709 and NCT01253642).

In preclinical studies, inhibition of DNMTs, DNA methyltransferases, could re-sensitize ARPI-resistant neuroendocrine-like PCa cell lines [27,99], suggesting that the development of DNMT inhibitors may be an attractive therapeutic strategy for NEPC. Notably, the DNMT inhibitors such as decitabine and azacytidine are already approved by the FDA for the treatment of myelodysplastic syndromes and could therefore be re-purposed to NEPC. However, in Phase II clinical trials in CRPC, DNMT inhibitors did not show a strong efficacy of anti-tumor activity [100,101]. Currently, a clinical trial in combination of DNMT inhibitor decitabine with enzalutamide in mCRPC patients has been just launched this year (NCT03709550). In deeded, future clinical trials will be needed to assess the efficacy of DNMT-directed therapies in NEPC patients.

Conclusion and Perspective

Numerous evidences have demonstrated that epigenetic and transcriptional dysregulation is central to the emergence and maintenance of lethal NEPC. Aberrant activities of master epigenetic regulators, such as DNMT1 and EZH2 and KDMs, as well as master transcription factors, such as N-Myc, facilitate chromatin remodeling to support the activation of lineage plasticity pathways under potent AR therapy. These epigenetic changes are increased, in part, through metabolic reprogramming. The dependency of NEPC tumors on the epigenetic and transcriptional machinery provides an excellent opportunity to develop effective therapeutical intervention. Although epigenetic therapy seems to be promising, still, numerous challenges remain with respect to patient responses, timing, and combination with ARPIs and/or immunotherapy to refine clinical outcomes. Moreover, biomarker-driven treatment strategy for NEPC is urgent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
2. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J Clin 1972;22:232-40.
3. Knudsen KE, Scher HI. Starving the addiction: New opportunities for durable suppression of AR signaling in prostate cancer. Clin Cancer Res 2009;15:4792-8.
4. Hotte SJ, Saad F. Current management of castrate-resistant prostate cancer. Curr Oncol 2010;17(Suppl 2):S72-9.
5. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424-33.
6. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138-48.
7. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, et al. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995;9:401-6.
8. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298-305.
9. Kong D, Sethi S, Li Y, Chen W, Sakr WA, Heath E, et al. Androgen receptor splice variants contribute to prostate cancer aggressiveness through induction of EMT and expression of stem cell marker genes. Prostate 2015;75:161-74.
10. Ferone G, Song JY, Sutherland KD, Bhaskaran R, Monkhorst K, Lambooij JP, et al. SOX2 Is the Determining Oncogenic Switch in Promoting Lung Squamous Cell Carcinoma from Different Cells of Origin. Cancer Cell 2016;30:519-32.
11. Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011;1:487-95.
12. Mosquera JM, Beltran H, Park K, MacDonald TY, Robinson BD, Tagawa ST, et al. Concurrent AURKA and MYCN gene amplifications are harbingers of lethal treatment-related neuroendocrine prostate cancer. Neoplasia 2013;15:1-0.
13. Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, et al. N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. Cancer Cell 2016;30:563-77.
14. Zhang Y, Zheng D, Zhou T, Song H, Hulsurkar M, Su N, et al. Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2-TSP1 pathway in prostate cancers. Nat Commun 2018;9:4080.
15. Abrahamsson PA, Dizeyi N, Alm P, di Sant'Agnese PA, Deftos LJ, Aumüller G. Calcitonin and calcitonin gene-related peptide in the human

prostate gland. Prostate 2000;44:181-6.
16. Qi J, Nakayama K, Cardiff RD, Borowsky AD, Kaul K, Williams R, et al. Siah2-dependent concerted activity of HIF and FoxA2 regulates formation of neuroendocrine phenotype and neuroendocrine prostate tumors. Cancer Cell 2010;18:23-38.
17. Eisinger-Mathason TS, Simon MC. HIF-1alpha partners with FoxA2, a neuroendocrine-specific transcription factor, to promote tumorigenesis. Cancer Cell 2010;18:3-4.
18. Zhang X, Coleman IM, Brown LG, True LD, Kollath L, Lucas JM, et al. SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. Clin Cancer Res 2015;21:4698-708.
19. Bishop JL, Thaper D, Vahid S, Davies A, Ketola K, Kuruma H, et al. The master neural transcription factor BRN2 Is an androgen receptor-suppressed driver of neuroendocrine differentiation in prostate cancer. Cancer Discov 2017;7:54-71.
20. Zou M, Toivanen R, Mitrofanova A, Floch N, Hayati S, Sun Y, et al. Transdifferentiation as a mechanism of treatment resistance in a mouse model of castration-resistant prostate cancer. Cancer Discov 2017;7:736-49.
21. Yu X, Cates JM, Morrissey C, You C, Grabowska MM, Zhang J, et al. SOX2 expression in the developing, adult, as well as, diseased prostate. Prostate Cancer Prostatic Dis 2014;17:301-9.
22. Mounir Z, Lin F, Lin VG, Korn JM, Yu Y, Valdez R, et al. TMPRSS2:ERG blocks neuroendocrine and luminal cell differentiation to maintain prostate cancer proliferation. Oncogene 2015;34:3815-25.
23. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644-8.
24. Kim S, Thaper D, Bidnur S, Toren P, Akamatsu S, Bishop JL, et al. PEG10 is associated with treatment-induced neuroendocrine prostate cancer. J Mol Endocrinol 2019;63:39-49.
25. Akamatsu S, Wyatt AW, Lin D, Lysakowski S, Zhang F, Kim S, et al. The Placental Gene PEG10 Promotes Progression of Neuroendocrine Prostate Cancer. Cell Rep 2015;12:922-36.
26. Rapa I, Ceppi P, Bollito E, Rosas R, Cappia S, Bacillo E, et al. Human ASH1 expression in prostate cancer with neuroendocrine differentiation. Mod Pathol 2008;21:700-7.
27. Reina-Campos M, Linares JF, Duran A, Cordes T, L'Hermitte A, Badur MG, et al. Increased serine and one-carbon pathway metabolism by PKCλ/ι deficiency promotes neuroendocrine prostate cancer. Cancer Cell 2019;35:385-400.
28. Maina PK, Shao P, Liu Q, Fazli L, Tyler S, Nasir M, et al. c-MYC drives histone demethylase PHF8 during neuroendocrine differentiation and in castration-resistant prostate cancer. Oncotarget 2016;7:75585-602.
29. Guo H, Ci X, Ahmed M, Hua JT, Soares F, Lin D, et al. ONECUT2 is a driver of neuroendocrine prostate cancer. Nat Commun 2019;10:278.
30. Ku SY, Rosario S, Wang Y, Mu P, Seshadri M, Goodrich ZW, et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. Science 2017;355:78-83.
31. Svensson C, Ceder J, Iglesias-Gato D, Chuan YC, Pang ST, Bjartell A, et al. REST mediates androgen receptor actions on gene repression and predicts early recurrence of prostate cancer. Nucleic Acids Res 2014;42:999-1015.
32. Tan HL, Sood A, Rahimi HA, Wang W, Gupta N, Hicks J, et al. Rb loss is characteristic of prostatic small cell neuroendocrine carcinoma. Clin Cancer Res 2014;20:890-903.
33. Tsai H, Morais CL, Alshalalfa M, Tan HL, Haddad Z, Hicks J, et al. Cyclin D1 loss distinguishes prostatic small-cell carcinoma from most prostatic adenocarcinomas. Clin Cancer Res 2015;21:5619-29.
34. Kim J, Jin H, Zhao JC, Yang YA, Li Y, Yang X, et al. FOXA1 inhibits prostate cancer neuroendocrine differentiation. Oncogene 2017;36:4072-80.
35. Cottard F, Asmane I, Erdmann E, Bergerat JP, Kurtz JE, Céraline J. Constitutively active androgen receptor variants upregulate expression of mesenchymal markers in prostate cancer cells. PLoS One 2013;8:e63466.
36. Isikbay M, Otto K, Kregel S, Kach J, Cai Y, Vander Griend DJ, et al. Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. Horm Cancer 2014;5:72-89.
37. Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell 2013;155:1309-22.
38. Yao JL, Madeb R, Bourne P, Lei J, Yang X, Tickoo S, et al. Small cell carcinoma of the prostate: An immunohistochemical study. Am J Surg Pathol 2006;30:705-12.
39. Epstein JI, Amin MB, Beltran H, Lotan TL, Mosquera JM, Reuter VE, et al. Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. Am J Surg Pathol 2014;38:756-67.
40. Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY, et al. The role of lineage plasticity in prostate cancer therapy resistance. Clin Cancer Res 2019;25:6916-24.
41. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
42. Nadal R, Schweizer M, Kryvenko ON, Epstein JI, Eisenberger MA. Small cell carcinoma of the prostate. Nat Rev Urol 2014;11:213-9.
43. Wang W, Epstein JI. Small cell carcinoma of the prostate. A morphologic and immunohistochemical study of 95 cases. Am J Surg Pathol 2008;32:65-71.
44. Furtado P, Lima MV, Nogueira C, Franco M, Tavora F. Review of small cell carcinomas of the prostate. Prostate Cancer 2011;2011:543272.
45. Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen CC, et al. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. Science 2017;355:84-8.
46. Davies AH, Beltran H, Zoubeidi A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. Nat Rev Urol 2018;15:271-86.
47. Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature 2017;547:453-7.
48. McKeithen D, Graham T, Chung LW, Odero-Marah V. Snail transcription factor regulates neuroendocrine differentiation in LNCaP prostate cancer cells. Prostate 2010;70:982-92.
49. Esposito S, Russo MV, Airoldi I, Tupone MG, Sorrentino C, Barbarito G, et al. SNAI2/Slug gene is silenced in prostate cancer and regulates neuroendocrine differentiation, metastasis-suppressor and pluripotency gene expression. Oncotarget 2015;6:17121-34.
50. Paranjape AN, Soundararajan R, Werden SJ, Joseph R, Taube JH, Liu H, et al. Inhibition of FOXC2 restores epithelial phenotype and drug sensitivity in prostate cancer cells with stem-cell properties. Oncogene 2016;35:5963-76.
51. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules 2016;21:965.
52. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res 2014;74:1272-83.
53. Aranda S, Mas G, Di Croce L. Regulation of gene transcription by Polycomb proteins. Sci Adv 2015;1:e1500737.
54. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 2006;125:301-13.
55. Clermont PL, Lin D, Crea F, Wu R, Xue H, Wang Y, et al. Polycomb-mediated silencing in neuroendocrine prostate cancer. Clin Epigenetics 2015;7:40.
56. Lee JK, Phillips JW, Smith BA, Park JW, Stoyanova T, McCaffrey EF, et al. N-myc drives neuroendocrine prostate cancer initiated from human

prostate epithelial cells. Cancer Cell 2016;29:536-47.
57. Chen L, Alexe G, Dharia NV, Ross L, Iniguez AB, Conway AS, et al. CRISPR-Cas9 screen reveals a MYCN-amplified neuroblastoma dependency on EZH2. J Clin Invest 2018;128:446-62.
58. Berger A, Brady NJ, Bareja R, Robinson B, Conteduca V, Augello MA, et al. N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer. J Clin Invest 2019;129:3924-40.
59. Xiang S, Zou P, Tang Q, Zheng F, Wu J, Chen Z, et al. HOTAIR-mediated reciprocal regulation of EZH2 and DNMT1 contribute to polyphyllin I-inhibited growth of castration-resistant prostate cancer cells *in vitro* and in vivo. Biochim Biophys Acta Gen Subj 2018;1862:589-99.
60. Aytes A, Giacobbe A, Mitrofanova A, Ruggero K, Cyrta J, Arriaga J, et al. NSD2 is a conserved driver of metastatic prostate cancer progression. Nat Commun 2018;9:5201.
61. Popovic R, Martinez-Garcia E, Giannopoulou EG, Zhang Q, Zhang Q, Ezponda T, et al. Histone methyltransferase MMSET/NSD2 alters EZH2 binding and reprograms the myeloma epigenome through global and focal changes in H3K36 and H3K27 methylation. PLoS Genet 2014;10:e1004566.
62. Asangani IA, Ateeq B, Cao Q, Dodson L, Pandhi M, Kunju LP, et al. Characterization of the EZH2-MMSET histone methyltransferase regulatory axis in cancer. Mol Cell 2013;49:80-93.
63. Shan J, Al-Muftah MA, Al-Kowari MK, Abuaqel SWJ, Al-Rumaihi K, Al-Bozom I, et al. Targeting Wnt/EZH2/microRNA-708 signaling pathway inhibits neuroendocrine differentiation in prostate cancer. Cell Death Discov 2019;5:139.
64. Ryu S, McDonnell K, Choi H, Gao D, Hahn M, Joshi N, et al. Suppression of miRNA-708 by polycomb group promotes metastases by calcium-induced cell migration. Cancer Cell 2013;23:63-76.
65. Saini S, Majid S, Shahryari V, Arora S, Yamamura S, Chang I, et al. miRNA-708 control of CD44(+) prostate cancer-initiating cells. Cancer Res 2012;72:3618-30.
66. Yang X, Chen MW, Terry S, Vacherot F, Chopin DK, Bemis DL, et al. A human- and male-specific protocadherin that acts through the wnt signaling pathway to induce neuroendocrine transdifferentiation of prostate cancer cells. Cancer Res 2005;65:5263-71.
67. Kim E, Kim M, Woo DH, Shin Y, Shin J, Chang N, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell 2013;23:839-52.
68. Lee ST, Li Z, Wu Z, Aau M, Guan P, Karuturi RK, et al. Context-specific regulation of NF-κB target gene expression by EZH2 in breast cancers. Mol Cell 2011;43:798-810.
69. Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, et al. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell 2011;20:457-71.
70. Nolan KD, Franco OE, Hance MW, Hayward SW, Isaacs JS. Tumor-secreted Hsp90 subverts polycomb function to drive prostate tumor growth and invasion. J Biol Chem 2015;290:8271-82.
71. Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, Wu JN, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. Nat Med 2015;21:1491-6.
72. Grau DJ, Chapman BA, Garlick JD, Borowsky M, Francis NJ, Kingston RE. Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. Genes Dev 2011;25:2210-21.
73. Bracken AP, Helin K. Polycomb group proteins: Navigators of lineage pathways led astray in cancer. Nat Rev Cancer 2009;9:773-84.
74. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell 2005;121:645-57.
75. Lapuk AV, Wu C, Wyatt AW, McPherson A, McConeghy BJ, Brahmbhatt S, et al. From sequence to molecular pathology, and a mechanism driving the neuroendocrine phenotype in prostate cancer. J Pathol 2012;227:286-97.
76. Raj B, Irimia M, Braunschweig U, Sterne-Weiler T, O'Hanlon D, Lin ZY, et al. A global regulatory mechanism for activating an exon network required for neurogenesis. Mol Cell 2014;56:90-103.
77. Metzger E, Wissmann M, Yin N, Müller JM, Schneider R, Peters AH, et al. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature 2005;437:436-9.
78. Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, et al. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. Cell 2006;125:483-95.
79. Chang B, Chen Y, Zhao Y, Bruick RK. JMJD6 is a histone arginine demethylase. Science 2007;318:444-7.
80. Cloos PA, Christensen J, Agger K, Maiolica A, Rappsilber J, Antal T, et al. The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. Nature 2006;442:307-11.
81. Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. Nat Rev Genet 2006;7:715-27.
82. Hsia DA, Tepper CG, Pochampalli MR, Hsia EY, Izumiya C, Huerta SB, *et al*. KDM8, a H3K36 me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation. Proc Natl Acad Sci U S A 2010;107:9671-6.
83. Wang LY, Kung HJ. Male germ cell-associated kinase is overexpressed in prostate cancer cells and causes mitotic defects via deregulation of APC/CCDH1. Oncogene 2012;31:2907-18.
84. Dimitrova E, Turberfield AH, Klose RJ. Histone demethylases in chromatin biology and beyond. EMBO Rep 2015;16:1620-39.
85. Wissmann M, Yin N, Müller JM, Greschik H, Fodor BD, Jenuwein T, et al. Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. Nat Cell Biol 2007;9:347-53.
86. Shin S, Janknecht R. Diversity within the JMJD2 histone demethylase family. Biochem Biophys Res Commun 2007;353:973-7.
87. Kahl P, Gullotti L, Heukamp LC, Wolf S, Friedrichs N, Vorreuther R, et al. Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence. Cancer Res 2006;66:11341-7.
88. Wang HJ, Pochampalli M, Wang LY, Zou JX, Li PS, Hsu SC, et al. KDM8/JMJD5 as a dual coactivator of AR and PKM2 integrates AR/EZH2 network and tumor metabolism in CRPC. Oncogene 2019;38:17-32.
89. Liang Y, Ahmed M, Guo H, Soares F, Hua JT, Gao S, et al. LSD1-mediated epigenetic reprogramming drives CENPE expression and prostate cancer progression. Cancer Res 2017;77:5479-90.
90. Coleman DJ, Sampson DA, Sehrawat A, Kumaraswamy A, Sun D, Wang Y, et al. Alternative splicing of LSD1+8a in neuroendocrine prostate cancer is mediated by SRRM4. Neoplasia 2020;22:253-62.
91. Urbanucci A, Barfeld SJ, Kytölä V, Itkonen HM, Coleman IM, Vodák D, et al. Androgen receptor deregulation drives bromodomain-mediated chromatin alterations in prostate cancer. Cell Rep 2017;19:2045-59.
92. Welti J, Sharp A, Yuan W, Dolling D, Nava Rodrigues D, Figueiredo I, et al. Targeting bromodomain and extra-terminal (BET) family proteins in castration-resistant prostate cancer (CRPC). Clin Cancer Res 2018;24:3149-62.
93. Asangani IA, Wilder-Romans K, Dommeti VL, Krishnamurthy PM, Apel IJ, Escara-Wilke J, et al. BET bromodomain inhibitors enhance efficacy and disrupt resistance to AR antagonists in the treatment of prostate cancer. Mol Cancer Res 2016;14:324-31.
94. Asangani IA, Dommeti VL, Wang X, Malik R, Cieslik M, Yang R, et al. Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 2014;510:278-82.

95. Isaacsson Velho P, Antonarakis ES. PD-1/PD-L1 pathway inhibitors in advanced prostate cancer. Expert Rev Clin Pharmacol 2018;11:475-86.
96. Zingg D, Arenas-Ramirez N, Sahin D, Rosalia RA, Antunes AT, Haeusel J, et al. The histone methyltransferase EZH2 controls mechanisms of adaptive resistance to tumor immunotherapy. Cell Rep 2017;20:854-67.
97. Cai C, He HH, Gao S, Chen S, Yu Z, Gao Y, et al. Lysine-specific demethylase 1 has dual functions as a major regulator of androgen receptor transcriptional activity. Cell Rep 2014;9:1618-27.
98. Sehrawat A, Gao L, Wang Y, Bankhead A 3rd, McWeeney SK, King CJ, et al. LSD1 activates a lethal prostate cancer gene network independently of its demethylase function. Proc Natl Acad Sci U S A 2018;115:E4179-E4188.
99. Gravina GL, Festuccia C, Millimaggi D, Dolo V, Tombolini V, de Vito M, et al. Chronic azacitidine treatment results in differentiating effects, sensitizes against bicalutamide in androgen-independent prostate cancer cells. Prostate 2008;68:793-801.
100. Sonpavde G, Aparicio AM, Zhan F, North B, Delaune R, Garbo LE, et al. Azacitidine favorably modulates PSA kinetics correlating with plasma DNA LINE-1 hypomethylation in men with chemonaïve castration-resistant prostate cancer. Urol Oncol 2011;29:682-9.
101. Thibault A, Figg WD, Bergan RC, Lush RM, Myers CE, Tompkins A, et al. A phase II study of 5-aza-2'deoxycytidine (decitabine) in hormone independent metastatic (D2) prostate cancer. Tumori 1998;84:87-9.