Neuroendocrine Differentiation in Prostate Cancer: Emerging Biology, Models, and Therapies

Loredana Puca,^{1,2} Panagiotis J. Vlachostergios,² and Himisha Beltran^{1,2}

¹Englander Institute for Precision Medicine, Weill Cornell Medicine and New York-Presbyterian Hospital, New York, New York 10021

²Division of Medical Oncology, Weill Cornell Medicine, New York, New York 10021

Correspondence: hip9004@med.cornell.edu

Although a *de novo* clinical presentation of small cell neuroendocrine carcinoma of the prostate is rare, a subset of patients previously diagnosed with prostate adenocarcinoma may develop neuroendocrine features in later stages of castration-resistant prostate cancer (CRPC) progression as a result of treatment resistance. Despite sharing clinical, histologic, and some molecular features with other neuroendocrine carcinomas, including small cell lung cancer, castration-resistant neuroendocrine prostate cancer (CRPC-NE) is clonally derived from prostate adenocarcinoma. CRPC-NE therefore retains early prostate cancer genomic alterations and acquires new molecular changes making them resistant to traditional CRPC therapies. This review focuses on recent advances in our understanding of CRPC-NE biology, the transdifferentiation/plasticity process, and development and characterization of relevant CRPC-NE preclinical models.

Cellular plasticity plays a pivotal role in driving treatment resistance in cancer. In prostate cancer, a subset of patients initially diagnosed with prostate adenocarcinoma may acquire histologic features of small cell neuroendocrine carcinoma on metastatic biopsy during later stages of disease progression after developing resistance to traditional systemic therapies (Bluemn et al. 2017). These may encompass a spectrum ranging from adenocarcinoma with neuroendocrine differentiation, mixed histologies, and in extreme cases complete transformation to small cell carcinoma, which is morphologically indistinguishable from other small cell cancers (Epstein et al. 2014). Neuroendocrine

carcinoma cells in this setting typically show low or absent androgen receptor (AR) expression, express classical neuroendocrine markers, and may harbor genomic features common in other small cell carcinomas such as *TP53* and *RBI* loss (Beltran et al. 2016a). Clinically, castration-resistant neuroendocrine prostate cancer (CRPC-NE) is associated with aggressive disease, relative resistance to AR-directed therapeutics, and sensitivity to platinum-based chemotherapy. Recent advances in the molecular characterization of CRPC-NE combined with preclinical observations have provided biologic insights into the evolutionary process and have informed the development of new therapeutics

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targets for this aggressive subset of advanced prostate cancer.

NEUROENDOCRINE CELLS IN BENIGN PROSTATE AND PROSTATE CANCER

Neuroendocrine cells (NEs) in the normal prostate gland were first described by Pretl in 1944 as the argentaffin basal cells (Kazzaz 1974). They exist among the prostate-specific antigen (PSA) producing luminal cells and the prostate basal cells, representing only a minor fraction of the epithelial compartment (<1%) (Parimi et al. 2014). The exact function of these normal prostatic NEs is not well understood, but they may play a role in supporting the growth of surrounding basal and luminal cells through the secretion of neuropeptides (e.g., calcitonin, serotonin, adrenomedullin, bombesin, and others). In the normal prostate gland, NEs lack the proliferation marker Ki67, appear to be postmitotic, and express antiapoptotic protein Bcl-2 (Bonkhoff et al. 1991, 1995; Nakada et al. 1993).

De novo small cell neuroendocrine carcinoma of the prostate is rare, and these cases are hypothesized to arise from these normal NEs or a multipotent stem cell located within the prostate gland based on the high expression of CD44 (Simon et al. 2009) and other genes related to stemness such as OCT-3/4 and Bmi (Patrawala et al. 2006). Several studies have suggested that NEs can help sustain surrounding prostate adenocarcinoma cellular growth through a paracrine mechanism via their secretory products (i.e., bombesin, serotonin [5-HT], neuron-specific enolase [NSE], a thyroid-stimulating-like peptide [thyroid-stimulating hormone, TSH], somatostatin [SST], parathyroid hormone-related protein [PTHrP]). For instance, bombesin has been shown to increase prostate cancer proliferation (Jongsma et al. 2000) and prevent apoptosis of prostate cancer cells in vitro (Salido et al. 2000). Receptors for various NE products such as serotonin (5HT1a) (Abdul et al. 1994), bombesin/gastrin-releasing peptide (GRP), gastrin-releasing peptide receptor (GRPR) (Markwalder and Reubi 1999), and somatostatin (SSTR1-5) (Dizeyi et al. 2002) are present in both benign prostate and prostate tumors.

INDUCERS OF THE NEUROENDOCRINE PHENOTYPE IN PROSTATE CANCER

A number of signaling molecules have been shown to trigger the development of neuroendocrine features in prostate adenocarcinoma cells in vitro. Androgen-deprivation therapy (ADT) affects the expression of epithelial markers with a decrease in E-cadherin and gain of mesenchymal markers, such as N-cadherin, vimentin, Zeb1, Twist1, and Snail2 (Sun et al. 2012), toward an epithelial-mesenchymal transition (EMT)-like state. LNCaP cells transfected with Snail undergo neuroendocrine differentiation with neurite-like morphologic characteristics and increased levels of classical NE markers such as neuron specific enolase (NSE) and chromogranin A; subsequent knockdown of Snail in these cells can revert this phenotype (McKeithen et al. 2010). Similar to ADT, loss or silencing of the AR itself with small interfering RNA (siRNA) may also trigger NE properties to LNCaP cells (Wright et al. 2003).

Hypoxia is a characteristic feature of advanced solid tumors and strongly associates with malignant tumor progression and resistance to chemotherapy. Studies have suggested that hypoxia facilitates the malignancy of prostate adenocarcinoma cells by increasing androgen-independence (Yamasaki et al. 2013). Other reports show that hypoxia can induce neuroendocrine programs through down-regulation of Notch signaling (Danza et al. 2012). Hypoxia down-regulates both PTEN and REST in prostate cancer epithelia inducing expression of neuronal genes and microRNAs (miRNAs) associated with neuroendocrine reprogramming (Liang et al. 2014).

The Wnt pathway may also play a role in neuroendocrine transformation of prostate cancer cells through activation of the Wnt pathway effector β -catenin. β -catenin induces neuroendocrine features in LNCaP cells (Ciarlo et al. 2012). LNCaP cells transfected with Wnt-11 resemble LNCaP cells grown in an androgen-depleted environment. Cells with Wnt-11 overexpression display high levels of the NE markers NSE and ASCL1. LNCaP cells in androgen-free medium and LNCaP cells transfected with Pro-

tocadherin-PC (PCDH-PC) show higher levels of luciferase β -catenin and expression of Wnt target genes (Uysal-Onganer et al. 2010).

Other inducers of the neuroendocrine phenotype in vitro include MnSOD (Quiros-Gonzalez et al. 2011; Courel et al. 2014), hASH-1 (Rapa et al. 2013), HB-EGF (Adam et al. 2002), RPTP α (Zhang et al. 2003), and cAMP (Cox et al. 1999). Overall these studies provided early insights into the appearance of neuroendocrine characteristics from adenocarcinoma cells.

REACTIVATION OF STEM CELL, DIFFERENTIATION AND NEURONAL PATHWAYS IN CRPC-NE

During progression toward the neuroendocrine state, multiple molecular programs are activated conferring neuronal and stem-like characteristics as shown by the CD49f High signature defined by the Witte group. Through the characterization of multiple epithelial cell populations in the human prostate, including basal stem-cell-like prostate cancer cells (CD49f Hi), they found that the small cell/NE prostate cancer transcriptional program is similar to the CD49f Hi program with overexpression of NOTCH, FGFR, WNT pathways (Smith et al. 2015).

The retrotransposon-derived gene *PEG10* (paternally expressed 10), a gene normally expressed during placental development is highly expressed in CRPC-NE patient tumors and the LTL331R transdifferentiation patient-derived xenograft (PDX) model (described below). *PEG10* promotes cell-cycle progression from G0/G1 in the context of *TP53* loss and regulates Snail expression via TGF- β signaling to promote invasion. LNCaP cells grown in ligand-depleted media, or treated with enzalutamide, results in *PEG10* overexpression that is reversible by adding a synthetic androgen (Akamatsu et al. 2015).

N-MYC (*MYCN*) is an oncogenic transcription factor in the *MYC* gene family that includes *MYC*, *MYCL*, and *MYCN*, frequently dysregulated in a number of human cancers (Dang 2012). N-MYC is important for neuronal development and drives proliferation of granule neuron pre-

cursors derived from neuronal progenitor cells. Overexpression and amplification of N-MYC have been implicated in driving a subset of neuroblastoma, small cell lung cancer, central nervous system (CNS) tumors (Brodeur et al. 1984; Knoepfler et al. 2002), and more recently neuroendocrine prostate cancer. In prostate cancer, N-MYC suppresses AR signaling and regulates the CRPC-NE program through transcriptional regulation and cooperation with Aurora kinase A (*AURKA*), *EZH2*, and other cofactors (Beltran et al. 2011; Dardenne et al. 2016; Lee et al. 2016).

The repressor element-1 silencing transcription factor (*REST*), also known as neuron restrictive silencing factor (*NRSF*), silences the expression of neuron-specific protein coding genes and miRNAs in nonneuronal cells (Ballas et al. 2005). *REST* down-regulation is observed in up to 50% of CRPC-NE tumors (Lapuk et al. 2012). LNCaP cells transfected with si-*REST* overexpress neuronal-associated genes as *Syn1*, *NTRK3*, *BDNF*, and *Grin2A* (Svensson et al. 2014). *REST* is a master regulator of hypoxia-induced genes and essential for hypoxia-induced neuroendocrine differentiation of prostate cancer cells (Lin et al. 2016). Recent studies evaluating 155 castration-resistant prostate cancer (CRPC) metastases from 50 patients, 24 prostate cancer PDX models (LuCaP), and a separate cohort of castration-resistant adenocarcinoma (CRPC-Adeno) and CRPC-NE patients identified a splicing form of *REST* induced by the serine/arginine-repetitive matrix 4 (*SRRM4*) in CRPC-NE. *SRRM4* was capable of promoting neuroendocrine differentiation generating an inactive splice variant form of *REST*, and these effects were further enhanced by loss-of-function of *TP53* or *RB1* (Zhang et al. 2015; Li et al. 2017). Interleukin-6 expression has also been reported as a suppressor of *REST* and is involved in resistance to the AR antagonist enzalutamide (Zhu et al. 2014).

The POU-domain transcription factor *BRN2* (*POU3F2*), a master regulator of neuronal differentiation, is highly expressed in AR indifferent preclinical models of enzalutamide resistance and CRPC-NE tumors (Bishop et al. 2016). *BRN2* is directly transcriptionally repressed by the AR. Therefore, in the setting of loss of AR

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signaling, BRN2 is up-regulated. BRN2 is capable of driving expression of SOX2 and promoting the neuroendocrine phenotype (Bishop et al. 2016).

LNCAP-AR infected with short hairpins against RB1 and TP53 also showed up-regulation of SOX2 together with increased expression of basal markers (CK5, CK14, and TP63) and neuroendocrine markers (SYP, CHGA) (Mu et al. 2017). Complete silencing of SOX2 in this LNCAP-AR shRB1/TP53 tumor model restores the sensitivity to enzalutamide in vivo, suggesting that SOX2 is a driver of lineage plasticity. The forkhead box protein A2 (FOXA2) has been shown to be up-regulated in mouse models that recapitulate CRPC-NE phenotype NMYC/AKT1 and TRAMP (Lee et al. 2016; Chiaverotti et al. 2008). In the TRAMP model, FOXA2 cooperates with hypoxia-inducible factor 1 alpha (HIF1 alpha) to induce genes as HES6, SOX9, and KDM3A involved in neuroendocrine differentiation (Qi et al. 2010). FOXA2 has been found highly expressed in 75% of small cell neuroendocrine prostate cancer patients analyzed by immunohistochemistry suggesting that it could be a marker for neuroendocrine tumors (Park et al. 2017) (Table 1).

GENOMIC ALTERATIONS IN CRPC-NE

Mutation or deletions involving the *RB1* and *TP53* genes are commonly observed in high-grade/small cell neuroendocrine carcinomas and are universally lost in small cell lung cancers (George et al. 2015). The combination of *RB1* and *TP53* loss can drive a small cell phenotype in lung and prostate cancer models (Greenberg et al. 1995; Gingrich et al. 1996; Zhou et al. 2006; Meder et al. 2016), suggesting they are important factors in the pathogenesis of NE tumors. *RB1* and *TP53* alterations are enriched in patients with CRPC-NE compared with castration-resistant prostate adenocarcinoma (Hansel et al. 2009; Williamson et al. 2011; Tan et al. 2014). In a cohort of 81 patients with CRPC (51 CRPC-Adeno, 30 CRPC-NE), *RB1* loss was present in 70% of CRPC-NE versus 32% CRPC-Adeno, mutation or deletion of *TP53* in 66.7% of CRPC-NE versus 31.4% of CRPC-Adeno (Beltran et al. 2016a). Recent analysis of

the International Stand Up to Cancer (SU2C)-Prostate Cancer Foundation (PCF) Dream Team advanced prostate cancer patient cohort showed that these alterations are also present in a subset of CRPC-Adeno (Robinson et al. 2015; Armenia et al. 2018). Analyses of other patient cohorts, including the ones from Johns Hopkins and University of Michigan, has shown that *RB1* loss is correlated with cyclin D1 protein down-regulation and overexpression of p16 (Tsai et al. 2015). High p16/CCND1 ratio was sufficient to distinguish prostatic small-cell carcinomas from adenocarcinomas (Tsai et al. 2015). *TP53* alterations are also frequently associated with *PTEN* loss in CRPC patients (23% co-occurrence) (Robinson et al. 2015). Loss of *PTEN* and *TP53* has been associated with abiraterone resistance and progression towards a small cell/neuroendocrine phenotype in genetically engineered (GEM) models (Zou et al. 2017).

The deubiquinating enzyme CYLD (cylindromatosis) is deleted in up to half of CRPC-NE (Beltran et al. 2016a). This enzyme has been reported as negative regulator of NF- κ B activation acting as tumor suppressor (Harhaj and Dixit 2011). CYLD is a crucial regulator of diverse cellular processes, such as immune responses, inflammation, death, and proliferation (Massoumi et al. 2010; Sun et al. 2010), and in prostate cancer, loss of CYLD has been correlated with decreased AR messenger RNA (mRNA) expression. Although silencing CYLD showed a decrease in expression of AR-signaling genes, dysregulation of CYLD was not sufficient to induce the transdifferentiation of LNCaP cells, suggesting that additional factors are required (Beltran et al. 2016a).

The *TMPRSS2-ERG* gene rearrangement, an early event in prostate cancer, is present in ~50% of CRPC-NE (Tomlins et al. 2005; Mertz et al. 2007; Mosquera et al. 2009; Lotan et al. 2011), at a similar frequency as prostate adenocarcinoma. As opposed to *TMPRSS2-ERG* fusion-positive prostate adenocarcinomas in which ERG protein is overexpressed (Tomlins et al. 2005; Park et al. 2014), ERG may not be overexpressed in *TMPRSS2-ERG*-positive CRPC-NE tumors as ERG protein expression is driven by its androgen-driven partner *TMPRSS2*, and

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Table 1. Summary of reported genes and pathways implicated in CRPC-NE progression

Key pathway/ gene	Summary of findings	Reference(s)
<i>MYCN/AURKA</i>	<i>MYCN/AURKA</i> (Aurora kinase A) are frequently overexpressed/amplified in CRPC-NE. LNCaP cells stably transfected with N-MYC show enhanced in vitro sensitivity to the Aurora kinase inhibitors	Beltran et al. 2011; Dardenne et al. 2016, Lee et al. 2016
<i>EZH2</i>	<i>EZH2</i> (Enhancer of zeste homolog 2) mRNA is twofold higher in the CRPC-NE patient cohort compared to CRPC-Adeno and interacts with AR and NMYC regulating the NMYC and AR transcriptional programs. <i>EZH2</i> inhibitor restores enzalutamide sensitivity in models of lineage plasticity (LNCaP-AR sh-RB1/sh-TP53, TKO, DKOCr)	Beltran et al. 2016a; Dardenne et al. 2016, Clermont et al. 2015; Mu et al. 2017
<i>PEG10</i>	<i>PEG10</i> (Paternally Expressed 10) is overexpressed in CRPC-NE patient samples and in the LTL331R PDX. LNCaP cells grown in ligand-depleted media, or treated with enzalutamide, results in increased <i>PEG10</i> expression	Clermont et al. 2015; Akamatsu et al. 2015
<i>PTEN/AKT pathway</i>	<i>PTEN</i> deletions are present in ~50%–60% of CRPC-NE patients. Activation of AKT by IGF-1 leads to neuroendocrine differentiation in LNCaP and DU145 prostate cancer cell lines	Wu et al. 2007; Tan et al. 2014
<i>TMPRSS2-ERG</i>	<i>TMPRSS2-ERG</i> gene rearrangement is reported in ~50% of CRPC-NE cases. ADT in <i>TMPRSS2:ERG</i> positive prostate cells causes down-regulation of ERG expression and increases neuroendocrine gene expression	Tomlins et al. 2005; Mosquera et al. 2009; Lotan et al. 2011; Mounir et al. 2015
<i>RB1/TP53</i>	<i>RB1</i> loss and mutations or deletions of <i>TP53</i> are detected in the majority of CRPC-NE. <i>RB1</i> loss is highly correlated to Cyclin D1 protein down-regulation and overexpression of p16/CDKN2A. <i>RB1</i> and <i>TP53</i> loss support ADT resistance phenotype and lineage plasticity	Tan et al. 2014; Tsai et al. 2015; Beltran et al. 2016a; Ku et al. 2017; Mu et al. 2017
<i>SOX2/SOX11</i>	<i>SOX2</i> is up-regulated in <i>RB1</i> and <i>TP53</i> deleted tumors and it is required for lineage plasticity <i>SOX11</i> is up-regulated in CRPC-NE tumors and its down-regulation causes down-regulation of neuroendocrine markers	Mu et al. 2017; Zou et al. 2017
<i>BRN2</i>	<i>BRN2</i> or POU3F2 (POU Class 3 Homeobox 2) is highly expressed in CRPC-NE and metastatic CRPC-Adeno with low circulating PSA. <i>BRN2</i> drives the expression of the terminal neuroendocrine markers and <i>SOX2</i> and it is transcriptionally repressed by AR	Bishop et al. 2016
<i>CYLD</i>	<i>CYLD</i> (Cylindromatosis) is deleted in ~50% of CRPC-NE samples and its loss has been associated to decreased AR mRNA expression	Beltran et al. 2016a

Continued

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Table 1. *Continued*

Key pathway/ gene	Summary of findings	Reference(s)
<i>SPDEF</i>	SPDEF (SAM Pointed Domain Containing ETS Transcription Factor) is underexpressed and hypermethylated in CRPC-NE patient cohort. SPDEF overexpression in prostate epithelium reduces tumor cell proliferation in vivo and in vitro	Cheng et al. 2014; Beltran et al. 2016a; Tsui et al. 2016
<i>REST</i>	REST (RE1 silencing transcription factor) down-regulation is observed in 50% of CRPC-NE tumors and it is essential for hypoxia-induced neuroendocrine differentiation of prostate cancer cells. LNCaP cells transfected with si-REST overexpress neuronal associated genes as <i>Syn1</i> , <i>NTRK3</i> , <i>BDNF</i> and <i>Grin2A</i>	Lapuk et al. 2012; Svensson et al. 2014
<i>SRRM4</i>	SRRM4 (Serine/arginine repetitive matrix 4) promotes neuroendocrine differentiation by generating an inactive splice variant form of REST	Li et al. 2016
<i>FOXA2</i>	The forkhead box protein A2 (FOXA2) is highly expressed in NMYC/AKT1 and TRAMP model and in 75% of patients with small cell neuroendocrine prostate cancer analyzed by immunohistochemistry. It cooperates with HIF1 α to induce genes involved in neuroendocrine differentiation as <i>HES6</i> , <i>SOX9</i> , <i>KDM3A</i>	Lee et al. 2016; Chiaverotti et al. 2008; Park et al. 2017 Qi et al. 2010

CRPC-NE, castration-resistant neuroendocrine prostate cancer; AR, androgen receptor; DKOCr, double knockout castrated; TKO, triple knockout; PDXs, patient-derived xenografts; IGF-1, insulin-like growth factor-1; ADT, androgen-deprivation therapy; CRPC-Adeno, castration-resistant adenocarcinoma; si, small interfering; sh, short-harpin; HIF1 α , hypoxia-inducible factor 1 α .

AR signaling is often low in CRPC-NE (Beltran et al. 2011). ERG is a transcription factor involved in embryonic development, cell cycle control, cell proliferation, differentiation, migration, and angiogenesis (Adamo and Ladomery 2016). Recent studies have implicated loss of ERG protein expression itself in *TMPRSS2*: ERG-positive prostate cells may be a key mediator of the dedifferentiation process important for CRPC-NE progression (Mounir et al. 2015).

EPIGENOMIC ALTERATIONS

In addition to genomic changes, significant differences have been reported between the DNA methylation profiles of CRPC-Adeno and CRPC-NE tumors with epigenetically dysregulated genes involved in neuronal and developmental programs (Beltran et al. 2016a; Kleb et al. 2016). A significantly hypermethylated and

underexpressed gene in the CRPC-NE cohort (Beltran et al. 2016a) was the tumor suppressor gene SPDEF (prostate-derived Ets factor). SPDEF overexpression in prostate epithelium has been shown to reduce tumor cell proliferation in vivo and in vitro (Cheng et al. 2014). SPDEF also modulates the epithelial-to-mesenchymal transition by up-regulating E-cadherin expression and down-regulating the expression of N-cadherin, SNAIL, SLUG, and vimentin (Tsui et al. 2016). In CRPC-NE, hypermethylation of SPDEF is associated with up-regulation of mesenchymal markers (Beltran et al. 2016a).

The histone methyltransferase EZH2 is highly expressed in CRPC-NE (more than two-fold increase compared with CRPC) (Beltran et al. 2011, 2016; Clermont et al. 2015, Kleb et al. 2016), and may be involved in coordinating the transcriptional pathways that are activated during neuroendocrine transdifferentiation

and, therefore, regulating tumor plasticity. Recent models with *Pten* and *Rb1* losses (PBCre4: *Pten*^{fl/fl}:*Rb1*^{fl/fl}, DKO) and *Pten* *Rb1* and *Trp53* losses (PBCre4:*Pten*^{fl/fl}: *Rb1*^{fl/fl}:*Trp53*^{fl/fl}, TKO) showed up-regulation of EZH2 together with appearance of neuroendocrine features. Silencing of EZH2 in cells derived by castration-resistant DKO tumors resulted in an increased expression of AR and luminal markers and a decrease of SYP-positive cells (Ku et al. 2017) (Table 1).

PRECLINICAL MODELS

Multiple preclinical models have been established to understand mechanisms of neuroendocrine transdifferentiation and develop therapeutic targets for CRPC-NE. Each model displays unique features that contribute to the comprehension of this phenotype.

Cell Lines

The NCI-H660 cell line (ATCC CRL-5813) was derived from a lymph node metastasis of a 63-yr-old patient diagnosed with small cell carcinoma. Initially classified as small cell lung cancer, this cell line was later reclassified as prostate origin based on the presence of the prostate cancer-specific *TMPR22-ERG* gene fusion (Mertz et al. 2007). NCI-H660 cells lack AR expression and express classical neuroendocrine markers (SYP, CD56, NSE). A number of studies have used the NCI-H660 cell line to test gene expression profiles and therapies both in vitro and in vivo. For instance, NCI H660 was used to study the function of *TP53* deletion/mutation in NEPC. Knocking down P53 in NCI-H660 resulted in enhanced AURKA expression (Li et al. 2015). NCI-H660 shows sensitivity to EZH2 inhibitors compared with adenocarcinoma cell lines, and NCI-H660 xenografts are sensitive to AURKA inhibition (Beltran et al. 2011, 2016b).

The PC3 cell line was derived from a bone metastasis of a patient with prostate cancer (Kaighn et al. 1979) and has been proposed as a model of CRPC-NE (Tai et al. 2011). Multiple sublines of the PC3 cell line have been developed that show increases in metastatic ability when

transplanted in mice. Although derived from adenocarcinoma, PC3 cell line proliferation is independent of androgen, and PC3 lacks expression of AR or PSA mRNA/protein, expresses CD44 and neuroendocrine markers NSE and chromogranin A (Tai et al. 2011), and it is *PTEN* deficient. Therefore, PC3 has been used to address questions about the neuroendocrine phenotype in comparison with the androgen-sensitive LNCaP cell line (Marchiani et al. 2010).

22Rv1 is a cell line that was derived by serial passaging of the xenograft CWR22R, isolated from a patient with prostate adenocarcinoma with bone metastases after repeated tumor regression and relapse under castrated condition (Sramkoski et al. 1999). The 22Rv1 cell line expresses AR mRNA/protein and PSA at the mRNA level, but not at the protein level, and these cells have some overlapping features of neuroendocrine prostate cancer. Although they are androgen sensitive (Chlenski et al. 2001), when maintained under hypoxia for a few days these cells up-regulate the NE marker NSE and develop neurite-like structures. This cell line when implanted in mice expresses NE markers within highly hypoxic tumorigenic regions (Qi et al. 2010) (Table 2).

Mouse Models

The transgenic adenocarcinoma of the mouse prostate (TRAMP) model was derived from C57BL/6 inbred mice, expressing the rat probasin promoter (androgen and zinc dependent) that drives the expression of SV40 T-antigen (SV40-Tag) transgene. The SV40-Tag induces oncogenic progression by binding to and inactivating the *Trp53* and *Rb1* tumor suppressors. TRAMP mice develop tumors that metastasize frequently to the lymph nodes and lungs. High-grade PIN (prostatic intraepithelial neoplasia) is found in the prostates of this model after 10–16 wk. This model develops poorly differentiated neuroendocrine tumors at 24 wk (Greenberg et al. 1995; Gingrich et al. 1996). *Pten* loss increases the rate of cancer progression in the TRAMP model (Kwabi-Addo et al. 2001). TRAMP has been used for testing chemotherapeutics during prostate cancer progression (Tang et al. 2009). This model

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Table 2. Preclinical models of CRPC-NE

CRPC-NE models	Sources	Features	Reference(s)
NCI-H660 cell line (ATCC CRL-5813)	Lymph node metastasis	Loss of the AR locus on chromosome X. Lack of sensitivity to androgen deprivation treatment. Harbors <i>TMPRSS2-ERG</i> gene fusion, expresses synaptophysin, CD56, NSE	Mertz et al. 2007
PC3 (ATCC CRL-1435)	Bone metastasis	Androgen-independent, lacks AR and PSA messenger RNA (mRNA) and protein expression and is <i>PTEN</i> deficient. Expresses CD44 and neuroendocrine markers NSE and chromogranin A	Kaighn et al. 1979; Marchiani et al. 2010; Tai et al. 2011
22Rv1 (ATCC CRL-2505)	Serial passaging of the xenograft CWR22R after castration-induced tumor regression and relapse	Androgen-sensitive, expresses AR and PSA mRNA/protein. Up-regulation of the neuroendocrine marker NSE. Protrusion of neurite-like structures under hypoxic conditions	Sramkoski et al. 1999; Chlenski et al. 2001; Qi et al. 2010
TRAMP	C57BL/6 mice expressing the rat probasin driving the expression of SV40 large and small T antigens in prostatic epithelial cells	Poorly differentiated neuroendocrine tumors by 24 weeks of age	Greenberg et al. 1995; Gingrich et al. 1996
<i>p53</i> ^{PE-/-} ; <i>Rb</i> ^{PE-/-}	GEM model of <i>Rb1/Trp53</i> co-deletion from the mouse prostate epithelium	Poorly differentiated phenotype with neuroendocrine features by ~32 weeks of age	Zhou et al. 2006
PBCre4: <i>Pten</i> ^{fl/fl} : <i>Rb1</i> ^{fl/fl}	GEM model with knock out of <i>Pten</i> and <i>Rb1</i> in mouse prostate epithelium	Develops PIN lesions and adenocarcinoma within 12 weeks, synaptophysin positive cells appear by week 25. Median survival 38 weeks. Develops metastases in lymph node, liver, and lung	Ku et al. 2017
PBCre4: <i>Pten</i> ^{fl/fl} : <i>Rb1</i> ^{fl/fl} : <i>Trp53</i> ^{fl/fl}	GEM model with knock out of <i>Pten</i> , <i>Rb1</i> and <i>Trp53</i> in mouse prostate epithelium	Develops castration-resistant tumors, survival rate of 16 weeks not extended with castration. Low levels of AR and overexpression of EZH2, SOX2, chromogranin A and synaptophysin	Ku et al. 2017
<i>Nkx3.1</i> ^{CreERT2/+} ; <i>Pten</i> ^{flox/flox} ; <i>Trp53</i> ^{flox/flox} (Npp53 mice)	GEM model with <i>Trp53</i> and <i>Pten</i> deletion in mouse prostate epithelium	Develops features of CRPC-Adeno under castrate conditions. Treatment with abiraterone results in the appearance of neuroendocrine features. Low levels of AR and epithelial cytokeratins, high level of Ki67, and overexpression of synaptophysin	Zou et al. 2017

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Table 2. *Continued*

CRPC-NE models	Sources	Features	Reference(s)
<i>MYCN</i> -myr <i>AKT1</i>	Human prostate basal cells with NMYC induction and AKT1 activation implanted in NSG mice	Develops tumors at 6–10 weeks. Low levels of AR and low androgen-regulated genes, expresses chromogranin, synaptophysin	Lee et al. 2016
<i>T2-Cre^{+/+};Pten^{f/f};LSL-MYC^{+/+}</i>	GEM model with <i>MYCN</i> gene induction and <i>Pten</i> deletion	Highly invasive tumors display foci of AR-positive adenocarcinoma and poorly differentiated sarcomatoid foci. The NMYC gene expression signature derived from these tumors resembles the one of the CRPC-NE human cohort	Dardenne et al. 2016
LUCAP 49 PDXs	Omental metastasis	Androgen-insensitive, lacks expression of PSA or AR, increased expression of synaptophysin and NSE. Highly aggressive, rapid growth in mice, >75% of nuclei positive for Ki67	True et al. 2002
MDA PCA 144 PDXs	Pelvic exenteration	Intense staining of chromogranin, synaptophysin, CD56, and negative for AR, PSA, PAP, and AMACR. High proliferation index with Ki67 positivity in >90% of nuclei. <i>TMPRSS2:ERG</i> gene fusion is detected	Aparicio et al. 2011
WISH-PC2 PDXs	Transurethral resection of the prostate	Lacks AR, PSA, PSMA, expresses chromogranin A, NSE, and synaptophysin, mutated form of <i>TP53</i> , overexpression of the anti-apoptotic protein BCL2. High proliferative index.	Pinthus et al. 2000
LTL352 and LTL370 PDXs	Urethral metastasis (LTL352) and penile metastasis (LTL370)	Lacks AR and PSA expression, intense staining for chromogranin and synaptophysin. <i>TMPRSS2:ERG</i> gene fusion is detected	Lin et al. 2014
MSK-PCA4 Organoids	Pleural effusion	Small cell features with high proliferation index, negative for AR, positive for synaptophysin	Gao et al. 2014
OWCM CRPC-NE organoids	Lymph node, pelvic mass, liver, bone biopsies	Neuroendocrine features with small- to medium-sized round cells, fine chromatin pattern, and some nuclear molding. Lacks AR expression, expresses chromogranin, synaptophysin, and CD56.	Puca et al. 2017

CRPC-NE, castration-resistant neuroendocrine prostate cancer; AR, androgen receptor; NSE, neuron-specific enolase; GEM, genetically modified mouse; PIN, prostatic epithelial neoplasia; CRPC-Adeno, castration-resistant adenocarcinoma; NOD-SCID, nonobese diabetic severe combined immunodeficiency; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; PAP, Prostatic Acid Phosphatase; PSA, Prostate-specific antigen; AMACR, alpha-methylacyl CoA racemase; NSG, NOD scid gamma; TRAMP, transgenic adenocarcinoma of the mouse prostate.

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may be useful to study the evolution of the tumor and therapies that can be adopted at different stages of the disease, including CRPC-NE.

In a conditional knockout model ($p53^{PE-/-}$; $Rb^{PE-/-}$), *Trp53* and *Rb1* have been knocked out from the mouse prostate epithelium using a prostate-specific expression of a CRE recombinase under the direction *Arr2pb* promoter (Zhou et al. 2006). These mice develop PIN lesions in 8 wk and by week 32 they show a poorly differentiated phenotype with neuroendocrine features (Zhou et al. 2006). The appearance of this phenotype is strict to both *Trp53* and *Rb1* losses because inactivation of either *Trp53* and *Rb1* in mouse prostate generate PIN lesions with no further tumor development, suggesting that the synergistic inactivation of *Trp53* and *Rb1* may be needed for neuroendocrine differentiation in these mice. However, in more recently reported GEM models described below, either *Trp53* or *Rb1* in the setting of *Pten* loss may also develop neuroendocrine features, accelerated with an additional hit.

As reported by Ku et al. (2017), *PBCre4:Pten^{fl/f}:Rb1^{fl/f}* (DKO) mice developed PIN lesions by 12 wk, had shorter survival compared with single knockout models (38 wk vs. 48 wk), distant metastasis (lymph node lung and liver), and showed heterogeneous levels of AR and Cytokeratin 8 (CK8). Cells expressing the neuroendocrine marker synaptophysin appeared by 20–25 wk of age. Surgical castration of these mice extended survival by >48 wk, suggesting that they were ADT sensitive. DKO tumors that relapsed after castration showed a significant reduction of AR expression and spontaneous *Trp53* mutations (V173M, R282Q). This model highlighted *Rb1* loss as enhancer of lineage plasticity initiated by *Pten* loss.

The authors, as a consequence of spontaneous *Trp53* mutations found in DKO castrated tumors, developed another GEM model harboring *PBCre4:Pten^{fl/f}:Rb1^{fl/f}:Trp53^{fl/f}* (TKO mice). These TKO mice had a survival rate of 16 wk, developing a very aggressive phenotype with metastasis in lung, liver, and bone. Castration did not extend the survival rate, suggesting that these tumors were ADT insensitive. TKO tumors showed very low AR expression

and patchy synaptophysin positivity (Ku et al. 2017).

Recently, Zou and colleagues (2017) established inducible GEM models for CRPC-NE with *Trp53* and *Pten* loss, *Pten^{flox/flox}:Trp53^{flox/flox}* mice (NPp53 mice) using *Nkx3.1^{CreERT2}* as driver. This model has been compared with a previously described *Nkx3.1^{CreERT2/+}:Pten^{flox/flox}* mouse (NP mice) (Floc'h et al. 2012). NPp53 model as NP mice developed features of CRPC-Adeno in castrated conditions but treatment with the drug abiraterone, an androgen synthesis inhibitor, resulted in a significant reduction of tumor volume in NP mice, whereas castrated NPp53 tumor growth was not inhibited. In some NPp53 mice, abiraterone treatment supported the progression of the tumor, as well as the appearance of neuroendocrine features with reduced expression of AR, reduction of epithelial cytokeratins, high level of proliferation, and up-regulation of synaptophysin (Zou et al. 2017).

Lee and colleagues engineered human basal epithelial prostate cells with *AKT1*, a gene frequently activated in prostate cancer, including CRPC-NE (Conley-LaComb et al. 2013) and N-MYC by lentiviral transduction. N-MYC-AKT1 epithelial cells were mixed with mouse urogenital sinus mesenchyme (UGSM) and implanted subcutaneously in NSG. These mice developed tumors after 6–10 wk. These tumors consisted of high-grade adenocarcinoma with regions of neuroendocrine features, including high nuclear-to-cytoplasmic ratio, frequent mitotic figures with low levels of expression of AR, and the androgen-regulated genes *NKX3-1*, *KLK3*, and *TMPRSS2*, and expression of the neuroendocrine markers CHGA, SYP, NCAM1, and NSE. The AURKA inhibitor CD532 reduced N-MYC protein expression in this model, and substantially slowed tumor growth in vivo (Lee et al. 2016).

Dardenne et al. generated GEM mice that carry human *MYCN* gene integrated into the *ROSA26* (LSL-MYCN) locus and a *Tmprss2*-driven tamoxifen-activated Cre recombinase to drive its expression in luminal prostate cells. These mice also harbored a *Pten* conditional knockout allele. After induction with tamoxifen, N-MYC was overexpressed and, in the context

of *Pten* loss, led to focal mouse high-grade prostatic intraepithelial neoplasia (mHGPIN) at 3 mo postinduction. At 9 mo postinduction, N-MYC overexpressed tumors were highly invasive, displaying foci of AR-positive adenocarcinoma and poorly differentiated foci overexpressing the mesenchymal marker vimentin, no pancytokeratin expression, and variable levels of AR expression. The N-MYC gene expression signature derived from these tumors resembled the one of CRPC-NE human cohort (Dardenne et al. 2016).

Lineage Plasticity and Transdifferentiation

Clinical data, including genomic analyses of metastatic tumors, have supported an evolution of CRPC-NE from a prostate adenocarcinoma precursor. Recent preclinical studies have shed further light on the origin of CRPC-NE and the mechanisms driving this evolutionary process. Two concepts have been formulated by the authors supported by GEM mouse models: Ku et al. (2017) and Mu et al. (2017) define the concept of “lineage plasticity” as a transition state in which cells are in a multilineage-stem-like state that can be reverted or sealed by treatment pressure. Zou and colleagues define the “transdifferentiation” process in which a luminal adenocarcinoma cell transforms into an NE acquiring a new transcriptional program.

Ku and colleagues observed the existence of cellular heterogeneity with both luminal-like $Krt8^{high};Syp^{low}$ cells and neuroendocrine-like $Krt8^{low};Syp^{high}$ cells within DKO primary and metastatic tumors. They incorporated the Brainbow 2.1 lineage tracing allele into DKO mice, and observed that all the end-stage tumors were monocolored, suggesting that these heterogeneous DKO tumors, both primary and metastatic, were likely derived from a single neoplastic cell clone. Moreover, the early $Syp^{high};Krt8^{low}$ foci still expressed AR consistent with the derivation of $Krt8^{low};Syp^{high}$ cells from preexisting $Krt8^{high};Syp^{low}$ tumor. The authors defined the transcription factor SOX2 as main driver of this plasticity event because silencing of SOX2 in the LNCAP AR shRB1/TP53 tumors restores the

sensitivity to enzalutamide in vivo, as well as the reduction of neuroendocrine markers.

The GEM model described by Zou et al. (2017) with *Tpr53* and *Pten* losses (NPp53 mice) instead showed up-regulation of SOX11 and down-regulation of SOX2. Short Harpins RNA against SOX11 in NPp53 mice resulted in down-regulation of neuroendocrine markers as SYP and NSE. By analogy with neurogenesis, the authors reported that SOX2 and SOX11 were drivers of two different neuroendocrine-associated states in which SOX2 defined early events relative to lineage plasticity, whereas SOX11 was implicated in the final neuroendocrine differentiation process (Fig. 1). The NPp53 model elegantly showed the existence of the transdifferentiation process from a luminal/adenocarcinoma cell in a neuroendocrine, high proliferative cell. For this purpose, the authors used a lineage tracing in NPp53 mice with a R26R-yellow fluorescent protein (YFP) reporter allele expressed in luminal epithelial cells in adult prostate. With this system, the authors showed that SYP+ cells that arose after castration in NPp53 mice were all YFP-positive, demonstrating that neuroendocrine cells in CRPC-NE tumors are of luminal origin.

Patient-Derived Xenograft and Organoid Models

Patient-derived xenograft (PDX) models represent another resource to study the CRPC-NE phenotype. Several groups have developed and characterized prostate cancer PDXs from multiple metastatic sites.

The LUCAP 49 PDX was derived from an omental metastasis of a patient with poorly differentiated neuroendocrine cancer. It is androgen insensitive, lacks expression of PSA and AR, and overexpresses neuroendocrine markers. The tumor is highly aggressive and rapidly growing in mice with >75% of nuclei positive for Ki67 (True et al. 2002).

The MDA PCA 144 PDX was derived from the salvage pelvic exenteration specimen (radical resection of the rectum, bladder, and reproductive organs) of a patient with castrate-resistant prostate carcinoma with small cell carcinoma

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Figure 1. Schematic view of events leading to castration-resistant neuroendocrine prostate cancer (CRPC-NE). CRPC-NE arises as a resistant mechanism to androgen receptor-targeted therapy. Over time adenocarcinoma cells undergo a selective treatment pressure, acquiring multiple genomic (e.g., *RB1* and *TP53*) and epigenomic (e.g., DNA methylation and high *EZH2*) alterations together with activation of different pathways. The silencing transcription factor (REST). Cells acquire stem and neuronal characteristics associated with up-regulation of N-MYC, PEG10, and BRN2, SOX2, and SOX11. Cells start transitioning toward a mesenchymal state with up-regulation of VIM and SNAIL, drastically decreasing the expression of androgen receptor (AR) and androgen-regulated genes, such as prostate-specific antigen (PSA), and up-regulating classical neuroendocrine markers (e.g., SYP (synaptophysin), CHGA (chromogranin A), and neuron-specific enolase [NSE]). This ultimately results in increased proliferation index and mitogen signaling, including up-regulation of AURKA (Aurora Kinase A) and Ki67.

features. The tumor strongly expresses chromogranin, synaptophysin, and CD56, and lacks expression of AR, PSA, prostatic acid phosphatase (PAP), and P504S (α -methylacyl-CoA racemase, AMACR) (Aparicio et al. 2011), and the *TMPRSS2:ERG* gene fusion is detected by FISH. This tumor displays a high proliferation index with Ki67 positivity in >90% of nuclei.

The WISH-PC2 xenograft was derived from a transurethral resection of the prostate of a patient with Gleason score of 8 (3 + 5) prostatic adenocarcinoma with NE differentiation. The tumors resemble the ones of the patient with expression of NE tumor markers chromogranin A, NSE, and synaptophysin, mutated form of *TP53*, expression of antiapoptotic protein BCL2, high proliferative index, and absence of AR, PSA, PAP, prostate stem cell antigen (PSCA), and prostate-specific membrane antigen (PSMA). This tumor has been serially passaged in vivo and is able to grow in castration conditions (Pinthus et al. 2000).

The LTL352 and LTL370 xenografts originated by engrafting prostate tumors from a urethral (LTL352) and penile metastasis (LTL370) under the kidney capsule of NOD/SCID mice. Tumors engrafted in mice retained the expression of neuroendocrine markers CGHA and SYP, were immunonegative for AR and PSA expression, and the *TMPRSS2:ERG* gene fusion was detected. Interestingly, another similar adenocarcinoma PDX model, LTL331 after host castration (termed LTL331R posttreatment) showed retention of genomic features of adenocarcinoma but histologic changes of CRPC-NE, expression of NE markers, and loss of AR and AR signaling, including PSA expression after host castration. Similar to patients with CRPC-NE, LTL331R showed up-regulation of neuronal transcription factors, membrane ion receptors, secreted peptides, and up-regulation of epigenetic regulators (e.g., EZH2, CBX2) compared with the precastration adenocarcinoma LT331 model (Lin et al. 2014).

PDXs are advantageous as they retain the cellular heterogeneity, architectural and molecular characteristics of the original patient tumor. Although compared with GEM models they are immune deficient so they cannot be used to test

compounds that interfere with the tumor immune microenvironment, PDXs represent a valuable model to evaluate the efficacy of targeted therapies.

In 2014, Karthaus et al., Gao et al., and Chua et al. further impacted the development of patient relevant preclinical models in prostate cancer through organoid models derived from normal prostate and metastatic prostate cancer biopsies (Chua et al. 2014; Gao et al. 2014; Karthaus et al. 2014). Organoids from normal prostate are three-dimensional (3D) cultures that recapitulate the organ multicellular structures in vitro by forming the basal and luminal layers of the normal prostate. Lineage-tracing showed that luminal cells can form organoids and generate basal cells in culture. Organoids from patient needle biopsies are 3D cultures of cancer cells, which act as “micrometastases” in culture. The 3D environment is generally obtained by embedding organoids in an extracellular matrix that sustains cancer growth in 3D, and closely reflects the natural behavior of cancer tissue (Drost et al. 2016; Pauli et al. 2016). Patient-derived organoids retained the mutational profile of the corresponding original tumor and recapitulate the histological features of the tumor post-engraftment in mice (Gao et al. 2014). Seven organoid lines were generated from metastatic biopsies (MSK PCA1-7) in Gao et al., and one of them displayed neuroendocrine features (MSK PCA4). MSK PCA4 derives from a pleural effusion of a patient who received ADT, bicalutamide, and docetaxel treatments. It displays small cell features with high proliferation index, negative for AR, and positive for synaptophysin staining in both 3D culture and when transplanted in mice. This model also displays *PTEN* and *RB1* losses, and *TP53* is mutated (Gao et al. 2014). Recently, newly established CRPC-NE organoid models were developed by Puca et al. (2017). These models retain the histological and molecular features of the corresponding patient over passages in culture, and were used in high-throughput drug screenings to nominate novel potential therapeutic targets for CRPC-NE. These CRPC-NE models increase the number of in vitro models to study CRPC-NE pathogenesis and treatment hypotheses.

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EMERGING THERAPEUTIC TARGETS

Early sequencing studies led to the discovery of N-MYC and AURKA as frequently overexpressed in CRPC-NE compared with prostate adenocarcinoma (Beltran et al. 2011; Mosquera et al. 2013). AURKA is a cell cycle kinase mainly localized at centrosome-regulating mitosis entry and formation of the mitotic spindle (Dominguez-Brauer et al. 2015). Similar to neuroblastoma, N-MYC is stabilized by AURKA in a catalytic activity-independent fashion, and this interaction prevents N-MYC proteasomal degradation in human neuroblastoma (Otto et al. 2009). This complex can be targeted using allosteric Aurora-A inhibitors (Brockmann et al. 2013; Gustafson et al. 2014). N-MYC-overexpressing prostate cancer models show enhanced sensitivity to selected aurora kinase inhibitors, resulting in destabilization of N-MYC protein and reduction of tumor burden in vivo (Beltran et al. 2011; Dardenne et al. 2016; Lee et al. 2016). These initial observations led to a Phase II clinical trial of the AURKA inhibitor alisertib for patients with de novo or treatment-related CRPC-NE (NCT01799278) (Beltran et al. 2016b), as well as further investigation of additional strategies to drug N-MYC and the N-MYC-AURKA complex in CRPC-NE.

Beyond its role in concert with AURKA, N-MYC can also interact with AR and the histone methyltransferase EZH2 regulating the N-MYC and AR transcriptional program and driving neuroendocrine phenotype (Xu et al. 2012; Dardenne et al. 2016). Moreover, the expression of EZH2 is higher in CRPC-NE tumors compared with CRPC-Adeno (Beltran et al. 2016a), and the neuroendocrine cell line (NCI-H660) is more sensitive to EZH2 inhibitor than androgen-dependent LNCaP cells. EZH2 inhibitor treatment with 3-deazaneplanocin A (DZNep) resulted in AR expression and growth inhibition in AR (–) cell lines (Kleb et al. 2016). Hormone-naïve AR/PSA-positive adenocarcinoma PDXs (LTL331) that, on host castration, progress to a terminally differentiated CRPC-NE (LTL331R) show similar results with up-regulation of the epigenetic regulators EZH2 and CBX2 (Clermont et al. 2015). Cotreatment of castration-

resistant cells derived from DKO tumor with EZH2 inhibitor and enzalutamide increased AR expression and reduced neuroendocrine markers, suggesting the usage of EZH2 inhibitor to revert this lineage transformation (Ku et al. 2017). Phase 1 trials of EZH2 inhibitors are currently underway (NCT02082977, NCT01897571).

Bromodomain and extraterminal (BET) family proteins directly regulate the expression of MYC genes, and disruption of BET protein-binding silences MYC gene expression (Delmore et al. 2011). The inhibitor of BET, I-BET762, reduces MYC expression in LNCaP prostate cancer cell lines and in patient-derived tumor model with inhibition of cell growth and reduction of tumor burden in vivo (Wyce et al. 2013; Fu et al. 2015). BET inhibitors therefore may represent a potential approach and area of research for targeting N-MYC-driven CRPC-NE.

The high rate of *PTEN* loss that occurs in CRPC-NE and prior studies implicating AKT1 expression as a driver of CRPC-NE together with N-MYC, as well as the ability of *PTEN* loss alone in GEM models to induce CRPC-Adeno (Floc'h 2012; Zou et al. 2017), suggest that the inhibition of PI3K/AKT/mechanistic target of rapamycin (mTOR) pathway may be an effective approach to inhibit tumor progression to CRPC-NE. Curcumin, a dietary component that can inhibit PI3K, has been shown to inhibit PIN formation in TRAMP mice (Li et al. 2007). Multiple clinical trials have been developed to target this pathway in CRPC patients with no specific arm for CRPC-NE patients.

Recent advances in CRPC-NE biology have provided new insights toward the development of novel drug targets, including BRN2 and SRRM4. Developing specific inhibitors targeting the activity of SRRM4 or BRN2 during antiandrogen targeted therapy could represent novel approaches for CRPC-NE treatment and warrant further investigation.

New insights into the treatment of CRPC-NE have also come from small cell lung carcinoma studies. A recent study in small cell lung cancer (SCLC) identified the Delta-like ligand 3 (DLL3), a cell surface ligand for the Notch receptor, as highly overexpressed in SCLC patients and PDXs. An antibody targeting DLL3 linked to a

potent cytotoxin results in internalization and complete and durable responses in SCLC PDX models (Saunders et al. 2015), and has shown promising results in patients (Rudin et al. 2016). A Phase I clinical trial (NCT02709889) is now open to evaluate the effect of this antibody-conjugated toxin (Rovalpituzumab-Tesirine) in DLL3-expressing solid tumors, including a dedicated CRPC-NE arm.

CONCLUDING REMARKS

Recent genomic landscape studies combined with preclinical observations and the development of new model systems have provided new insights into mechanisms driving treatment resistance in prostate cancer. Neuroendocrine prostate cancer is a complex androgen-independent phenotype that may arise de novo or in later stages of prostate cancer progression as a mechanism of treatment resistance from an adenocarcinoma precursor. Ongoing efforts are focused on establishing targeted and cotargeting approaches based on the identified molecular drivers of CRPC-NE.

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