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INVITED REVIEW

Prostate Cancer

Lineage plasticity-mediated therapy resistance in prostate cancer

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Therapy resistance is a significant challenge for prostate cancer treatment in clinic. Although targeted therapies such as androgen deprivation and androgen receptor (AR) inhibition are effective initially, tumor cells eventually evade these strategies through multiple mechanisms. Lineage reprogramming in response to hormone therapy represents a key mechanism that is increasingly observed. The studies in this area have revealed specific combinations of alterations present in adenocarcinomas that provide cells with the ability to transdifferentiate and perpetuate AR-independent tumor growth after androgen-based therapies. Interestingly, several master regulators have been identified that drive plasticity, some of which also play key roles during development and differentiation of the cell lineages in the normal prostate. Thus, further study of each AR-independent tumor type and understanding underlying mechanisms are warranted to develop combinational therapies that combat lineage plasticity in prostate cancer.

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INTRODUCTION TO STANDARD THERAPIES AND RESISTANCE

As the number one diagnosed cancer and second leading cause of cancer death in American men,[1] prostate cancer (PCa) and effective therapies remain pressing clinical issues. Although prostate-specific antigen (PSA) screening has led to an overall decrease in the number of metastatic disease diagnoses, improved screening has also increased clinically indolent cancer diagnoses.[1,2] Earlier detection of potentially indolent disease can lead to unnecessary treatment and represents a significant economic burden.[3,4] This effect is magnified by a lack of improved survival for either indolent or metastatic disease with PSA screening,[2–5] and accompanied by unavoidable biochemical recurrence and disease progression.[6]

For earlier-stage prostate tumors, the standard of care can include watchful waiting, active surveillance, radical prostatectomy, or radiation therapies.[7] Further treatment for recurrence or disease progression can include continuous or intermittent hormonal therapies.[7] The androgen receptor (AR) is a frequent and well-established target of hormonal therapies such as androgen deprivation therapy (ADT).[8,9] Abiraterone, a potent cytochrome P450 17A1 (CYP17A1 or steroid 17α-monooxygenase) inhibitor,[10] and enzalutamide, a small molecule AR antagonist,[11] both lead to a significant reduction in AR activity,[9,12,13] but are eventually overcome in castration-resistant prostate cancer (CRPC). Next-generation AR-targeted therapies under development such as the CYP17 inhibitor Seviteronel (VT-464)[14] and the AR antagonists Apalutamide (ARN-509),[15,16] Darolutamide (ODM-201),[17,18] and EPI-001,[19] may further prolong CRPC recurrence,[20] but the current standard treatment options for recurrent disease are limited to chemotherapy,[21,22] immunotherapy,[7,23] and radiopharmaceutical therapy.[24,25] These relatively uniform androgen ablation and chemotherapy treatment options do not fully account for the heterogeneous genetic background between individual patient tumors at the time of initial therapy, or variable molecular changes in response to therapy. As such, the current treatment options and unavoidable therapy resistance represent significant barriers to improving patient outcomes.

Several well-established mechanisms of resistance to AR-targeted therapies which have been characterized can be divided into three main categories: restored AR signaling, bypassed AR signaling, and AR signaling independence.[6,26] AR pathway signaling can be restored by AR mutation or truncation that most commonly alters the ligand binding domain (LBD).[27–33] These mutations allow for receptor promiscuity and activation by alternative steroid hormones or AR antagonists such as enzalutamide.[33,34] Truncated mutants, as well as splice variants, that lack the LBD are constitutively active and resistant to many AR-targeted therapies.[35–38] In addition, steroid hormone synthesis genes are often upregulated after chemical castration to restore AR signaling.[20,39] AR amplification is also observed after AR-targeted therapy;[40–42] however, increased AR levels that contribute to disease progression are also observed independently of gene amplification.[43] Bypass of AR signaling is thought to occur through other steroid hormone receptors,[6] such as glucocorticoid receptor (GR), which is upregulated in prostate tumors posttherapy.[44–46] GR shares similar protein structure, DNA binding motifs, and transcriptional targets with AR, suggesting that it may compensate for AR and contribute to castration resistance.[26,44,47,48] Finally, a subset of CRPC appear to be

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AR-independent, with minimal to no AR expression.[49–51] These tumors may display neuroendocrine (NE) phenotypes, but are heterogeneous and remain to be systematically characterized.[52] New insights into AR-independent CRPC suggest that cell lineage plasticity is a driving factor in loss of AR and resistance to AR-targeted therapies,[53–55] which is the focus of this review.

PROSTATE CELL LINEAGES DURING DEVELOPMENT AND IN THE ADULT

An understanding of normal prostate development and well-regulated maintenance of tissue identity is useful in order to understand the changes that occur during tumorigenesis and gain of therapy resistance. Although the mature mouse prostate has distinct lobes in contrast to the lobe-less adult human prostate, many prostate-centric studies are performed in mice due to the developmental and tumorigenic similarities between the human and mouse prostate.[56] Notably, human and rodent prostate tissues can be recombined to generate functional prostate tissues, arguing for the applicability of mouse models to study prostate development and tumorigenesis.[56,57]

During normal prostate development in the mouse, several key factors regulate differentiation of the urogenital sinus (UGS), including androgens and the androgen-responsive transcription factor NK3 homeobox 1 (NKX3.1).[58,59] Most importantly, secreted androgens activate the mouse AR protein that is initially only expressed in the stromal cells of the urogenital sinus mesenchyme (UGM) and is required for paracrine signaling that initiates organ differentiation.[60–63] Soon after organ differentiation, morphological prostate development occurs through epithelial budding and proliferation that leads to bud elongation, lumen formation, branching morphogenesis, differentiation, and maturation.[58,59,64] In particular, epithelial AR expression does not occur until after budding and branching morphogenesis,[65–68] while initial expression of the NKX3.1 transcription factor occurs as early as 15.5 days post coitum (dpc) in the urogenital sinus epithelium (UGE) and is important for prostatic bud formation and ductal morphology.[69] Subsequent androgen-regulated expression of NKX3.1 in the UGE and in the luminal epithelial cells of the differentiated prostate is maintained by epithelial AR expression.[64,69]

Further morphological development requires paracrine signaling from stromal to epithelial cells that includes many pathways and regulatory factors such as transforming growth factor beta (TGFβ), fibroblast growth factor (FGF), bone morphogenetic protein (BMP), insulin-like growth factor (IGF), sonic hedgehog (SHH), wingless and int1 (WNT), NOTCH, homeobox genes, forkhead genes, and sex-determining region Y-box 9 (SOX9), as reviewed previously.[58,64] Loss of these pathways during prostate development generally contributes to organ defects, and dysregulation of these pathways in the fully differentiated prostate is also implicated in hyperplasia and cancer.[70]

Fully developed prostate tissue in both human and mouse can be divided into stromal and epithelial components, where the epithelial component consists of CK5/CK14/CD44/p63-positive basal cells, CK8/CK18/CD57/NKX3.1/AR-positive luminal cells, and rare NE cells that express markers such as synaptophysin (SYP), chromogranin A (CGA), and neuron-specific enolase (NSE).[50,51,56,71] In the mature prostate, androgen-dependent luminal cells line the lumen of mature prostatic ducts and produce prostatic secretory proteins, while AR-negative basal cells form a layer between the luminal cells and the basement membrane.[56] AR-negative NE cells dispersed throughout the basal layer are important for signaling and regulation of growth, differentiation, and function of the prostate.[51,56,72] Further differentiation of the UGE component during development into these cell types is tightly regulated by the transcription factor p63 in basal progenitor cells.[73,74] p63-positive basal progenitor cells undergo either symmetric divisions to generate two progeny basal cells, or asymmetric divisions to generate one basal and one luminal cell,[75] a process dependent on mitotic spindle orientation regulated by GATA binding protein 3 (GATA3).[76] This plasticity results in double-positive intermediates, a fourth epithelial cell type found in the basal compartment of the developed prostate, which express both luminal and basal markers and have yet to fully differentiate into the luminal cell fate.[76–79] In contrast, luminal epithelial cells do not exhibit the same plasticity but instead divide symmetrically to generate two progeny luminal cells.[75]

Interestingly, the plasticity exhibited by basal progenitor cells is thought to contribute to prostate regrowth and repair, as well as tumorigenesis.[74,75] Phosphatase and tensin homolog (*PTEN*) is a well-established tumor suppressor gene in PCa, deletion of which in mice recapitulates human PCa progression from prostatic intraepithelial neoplasia (PIN) to invasive adenocarcinoma.[80] *Pten* loss and subsequent transformation of luminal epithelial cells are sufficient for tumorigenesis in mice, as is *Pten* loss in basal epithelial cells.[75,81,82] However, transformed basal cells maintain the ability to divide asymmetrically and generate transformed luminal progeny that express the stem cell and NE factor SOX2 and also drive tumorigenesis in mice.[74,75,81] This ability to generate stem-like, transformed progenitor cells in the prostate implies that established prostate tumors may also rely on cell lineage dysregulation as a means of therapy resistance.

TRANSDIFFERENTIATION AS A MECHANISM OF THERAPY RESISTANCE

While the emergence of different cancer cell populations post-therapy is not a novel concept, there are multiple proposed mechanisms to explain this plasticity phenomenon. Due to the reliance on ADT and AR targeting in PCa, AR-negative cells such as NE cells are frequently implicated in PCa therapy resistance. Therapies that specifically target a subpopulation of tumor cells, such as ADT that targets AR-positive PCa cells, may generate selective pressure for existing AR-negative NE cells to perpetuate AR-independent tumor growth. However, the majority of evidence suggests a more likely second mechanism of clonal divergence and transdifferentiation from adenocarcinoma (**Figure 1**). In this case, adenocarcinoma cells can proliferate and generate progenitor adenocarcinoma cells, but also transdifferentiate into AR-independent NE cells, specifically after ADT or AR-targeted therapy.[49,52] Here, transdifferentiation is defined as the switch from one differentiated cell type to another, which may progress through an intermediate cell type that is not pluripotent and involves a discrete change in the gene expression program of the cell.[83] In support of this mechanism, similar patterns of genetic alterations are observed between adenocarcinoma and NE foci in mixed tumors, including transmembrane protease, serine 2-ETS-related gene (*TMPRSS2-ERG*) fusion,[84–87] tumor protein p53 (*TP53*) mutation,[88] and neuroblastoma-derived v-myc avian myelocytomatosis viral related oncogene (*MYCN)* and aurora kinase A (*AURKA*) gene amplification.[84] Importantly, castration of a patient-derived adenocarcinoma xenograft has been shown to develop a small-cell NE phenotype and maintains similar genetic alterations as the parental lesion.[89] A similar phenomenon has been observed in the CWR22,[90] PC-295 and PC-130,[91] and MDA PCa 144[92] xenograft models. In-depth analysis of serial tumor samples from 81 patients with clinically and histologically classified CRPC-adenocarcinoma

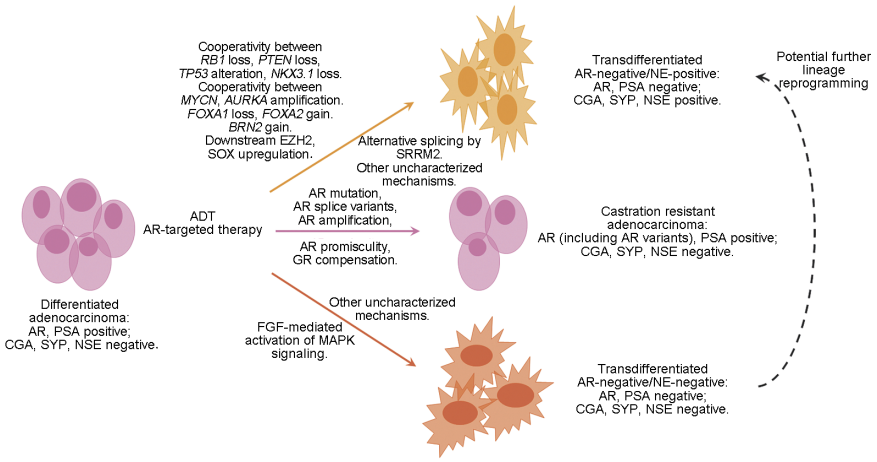

Figure 1: Model of therapy-induced prostate cancer cell lineage plasticity. Treatment of adenocarcinoma with ADT or AR-targeted therapy ultimately leads to therapy resistance through multiple mechanisms. Notably, adenocarcinoma cells can transdifferentiate into AR-negative/NE-negative or AR-negative/NE-positive tumor types or can rely on AR bypass signaling and AR mutation to develop castration resistant adenocarcinoma. ADT: androgen deprivation therapy; AR: androgen receptor; AURKA: aurora kinase A; BRN2: brain-specific homeobox/POU domain protein 2; CGA: chromogranin A; EZH2: enhancer of zeste homolog 2; FGF: fibroblast growth factor; FOXA1: forkhead box protein A1; FOXA2: forkhead box protein A2; GR: glucocorticoid receptor; NE: neuroendocrine; NKX3.1: NK3 homeobox 1; NSE: neuron-specific enolase; MAPK: mitogen-activated protein kinases; MYCN: neuroblastoma-derived v-myc avian myelocytomatosis viral related oncogene; PSA: prostate-specific antigen; PTEN: phosphatase and tensin homolog; RB1: retinoblastoma 1; SOX: sex-determining region Y-box; SRRM2: serine/arginine repetitive matrix 2; SYP: synaptophysin; TP53: tumor protein p53.

or CRPC-NE tumors also confirmed a pattern of alterations in serial samples to support a clonal divergent mechanism.[49]

Another class of AR-independent tumor posttherapy that also lacks NE differentiation is increasingly recognized in clinical tumor samples.[93,94] A study comparing two metastatic CRPC cohorts from 1998 to 2011 and from 2012 to 2016 highlights the existence of three unique tumor types: AR-positive/NE-negative, AR-negative/NE-positive, and less well-characterized AR-negative/NE-negative (or called double-negative) tumors.[95] These double-negative tumors possess heterogeneous mutational profiles and rely on increased FGF and mitogen-activated protein kinases (MAPK) signaling for tumor growth.[95] Notably, the AR-negative/NE-negative and AR-negative/NE-positive tumors have significantly increased in prevalence since 2012 and the widespread use of the second-generation AR-targeted therapies, suggesting that these tumors may also transdifferentiate in response to AR-targeting. Specifically comparing the cohort of patients with metastatic CRPC between 2012–2016 to the cohort from 1998–2011, the percentage of patients with AR-positive/NE-negative tumors has decreased by 25.1% to overall 63.3%, while the percentage of patients with AR-negative/NE-negative and AR-negative/NE-positive tumors has increased by 17.9% to overall 23.3% and 7.0% to overall 13.3%, respectively.[95] These data argue that lineage reprogramming represents a significantly growing population of therapy-resistant tumors in the era of AR-targeted therapies. Further study of the double-negative phenotype is warranted to determine if these tumors represent an intermediate transitional state between AR-positive/NE-negative and AR-negative/NE-positive tumors or a third fully differentiated tumor phenotype with a distinct molecular profile and clinical outcome.

CHARACTERISTICS OF AR-INDEPENDENT NEUROENDOCRINE PROSTATE CANCERS

The precise definition and characteristics of AR-negative/independent or neuroendocrine prostate cancer (NEPC) are actively being refined to reflect aspects of both morphological characterization and clinical behavior.[50] The Prostate Cancer Foundation Working Committee on NEPC has suggested that AR-negative/independent PCa and tumors with NE features should be referred to as AR-negative[96] and has also released guidelines for tumor subtype classifications.[72] Multiple tumor types fit this description, with subtle differences in morphology and clinical outcome. The proposed classifications for NEPC include six categories: (1) usual prostate adenocarcinoma with NE differentiation; (2) adenocarcinoma with Paneth cell NE differentiation; (3) carcinoid tumor; (4) small-cell carcinoma; (5) large-cell NE carcinoma; and (6) mixed (small or large cell) NE carcinoma–acinar adenocarcinoma.[72]

The morphological and clinical characteristics of each category have been outlined in detail previously.[72] Briefly, small-cell carcinoma is often positive for at least one NE marker such as SYP or CGA, negative for AR and PSA, and exhibits a sheet-like growth pattern with notably small tumor cells and mitotic figures.[51,97] Small-cell carcinomas often arise after hormone therapies[98,99] and exhibit rapid growth, poor prognosis, and therapy resistance.[100] Large-cell NE carcinomas, although exceedingly rare, display similar poor prognosis and often arise after ADT, but are characterized by notably large tumor cells with abundant cytoplasm and prominent nuclei.[101] In contrast to large- and small-cell carcinomas, rare carcinoid tumors are well-differentiated NE tumors without any components or markers of adenocarcinoma, and a relatively favorable prognosis.[50] It should be noted that only five verified cases of carcinoid tumors are found in the literature.[72] Adenocarcinoma with Paneth cell-like NE differentiation, recognized by foci of eosinophilic NE cells,[102] also has a favorable prognosis.[72] Usual prostate adenocarcinoma with NE differentiation represents tumors with typical adenocarcinoma morphology and markers that also express NE markers detected by immunohistochemistry, but that have no distinguishable difference in outcome.[72,103] In stark contrast, mixed NE carcinoma–acinar adenocarcinoma, with components of typical adenocarcinoma and distinct foci of NE carcinoma, is highly aggressive.[72,94] Interestingly, the degree of NE differentiation in these tumors increases in response to ADT.[104,105] Identification of this tumor type in particular further reinforces the idea of transdifferentiation as a mechanism of therapy resistance.

MECHANISMS OF TUMOR TRANSDIFFERENTIATION POSTTHERAPY

At the molecular level, several key factors are implicated in driving lineage plasticity and transdifferentiation of adenocarcinoma in response to hormone therapy and AR loss, and significant crosstalk exists between many drivers and downstream effectors (**Table 1**). AR and the androgen signaling pathway are known to protect the luminal epithelial cell lineage in AR-dependent LNCaP PCa cells, where loss of AR induces neuronal cell morphology and induces NSE expression.[106–108] The epithelial cell lineage transcription factor and AR pioneer factor forkhead box protein A1 (FOXA1) has been shown to inhibit interleukin-8 (IL-8) expression, thereby preventing MAPK/ extracellular signal-regulated kinase (ERK) signaling pathway-mediated progression to NE tumors.[109,110] Similarly, the AR-regulated *TMPRSS2-ERG* fusion has been shown to repress a neuronal gene signature,[111] although the exact mechanism and effect of additional mutations present in the cells studied remains unclear. In contrast, the NE cell transcription factor FOXA2 has been shown to cooperate with stabilized hypoxia-inducible factor 1 alpha subunit (HIF-1α) to promote NE tumor progression in the prostate.[112,113]

Frequent alterations found in CRPC are also implicated in promoting AR-negative/NE-positive tumor reprogramming. Analysis of human CRPC cohorts highlights that retinoblastoma 1 (*RB1*) copy number loss and *TP53* mutations frequently co-occur together in hormone therapy-resistant tumors,[49,114] and further study in mouse models has revealed a link with lineage plasticity. The well-established transgenic adenocarcinoma of the mouse prostate (TRAMP) model of PCa, which expresses SV40 large and small T antigens, has disrupted RB and p53 activity and ultimately progresses to a poorly differentiated state with NE characteristics.[52] A second model of PCa (LADY) consists of multiple lines that express solely the SV40 large T antigen and progresses similarly.[115–118] Interestingly, tumors in the TRAMP model also have progressively decreased expression of NKX3.1,[119] suggesting dysregulated prostate cell differentiation as a possible driver. Not surprisingly, loss of NKX3.1 in the developed mouse prostate leads to downregulation of genes associated with prostate differentiation.[120]

A recent study highlighted that *Rb1* and *Trp53* loss in the mouse prostate as well as in AR-dependent LNCaP cells and CWR22Pc-EP xenografts confers antiandrogen resistance through SOX2-mediated reprogramming to AR-independent NE-like cells.[54] In support of this, a second report emphasized the role of *Rb1*, *Trp53*, and *Pten* loss in lineage plasticity-mediated resistance, where combined loss of these three genes also resulted in reprogramming mediated by epigenetic modifier enhancer of zeste homolog 2 (EZH2) and lineage

Table 1: Key molecular drivers and effectors of androgen receptor-independent lineage reprogramming

Potential drivers	*Known downstream effectors*	*Final tumor cell outcomes*	*References*
TMPRSS2-ERG loss	Increased CD44+ cells; other uncharacterized factors	Repression of neuronal gene signature	Mounir *et al.*,[111] Li *et al.*[124]
FOXA1 loss	IL-8 expression	Increased MAPK/ERK signaling; progression to NE tumor phenotype	Kim *et al.*,[109] Zhao *et al.*[110]
FOXA2 gain	HIF-1α co-activation of HES6, SOX9, JMJD1A expression	Development of hypoxia-dependent NE tumor phenotype (in the TRAMP model)	Eisinger-Mathason *et al.*,[112] Qi *et al.*[113]
RB1/*TP53* loss	Enhanced SRRM4 function with concomitant AR inhibition; decreased NKX3.1; SOX2 activity; upregulated PEG10; other uncharacterized factors	Gain of neural cell differentiation genes; transdifferentiation to AR-negative/ NE-positive tumors; increased NE cell proliferation	Rickman *et al.*,[52] Ku *et al.*,[53] Mu *et al.*,[54] Lin *et al.*,[89] Grabowska *et al.*,[115] Masumori *et al.*,[116] Masumori *et al.*,[117] Yu *et al.*,[118] Bethel *et al.*,[119] Akamatsu *et al.*,[122] Li *et al.*[124]
RB1/*TP53*/*PTEN* loss	EZH2 and SOX2-mediated reprogramming	Lineage plasticity; transdifferentiation to AR-negative/NE-positive tumors	Ku *et al.*[53]
PTEN/*TP53* loss	SOX11-mediated reprogramming with decreased NKX3.1; other uncharacterized factors	Lineage plasticity; transdifferentiation to AR-negative/NE-positive tumors	Zou *et al.*,[55] Blee *et al.*,[121] Martin *et al.*[123]
NKX3.1 loss	FOXA1 and HOXB13 downregulation; G9a co-activation of UTY expression	Altered histone methylation; loss of differentiated prostate structures	Bethel *et al.*,[119] Dutta *et al.*[120]
SRRM4-mediated alternative splicing of neural cell differentiation genes	REST decrease and REST4 increase in the context of castration alone, RB1 loss, or TP53 loss	Gain of neural cell differentiation genes; altered cell morphology (LNCaP) in combination with AR inhibition; transdifferentiation to AR-negative/NE-positive cells	Li *et al.*,[124] Gopalakrishnan *et al.*,[133] Raj *et al.*[134]
BRN2 gain	SOX2 activity and co-regulation of SOX2 target genes	NE tumor progression	Bishop *et al.*,[125] Dailey *et al.*[126]
MYCN/AURKA amplification	EZH2-mediated reprogramming	NE tumor progression	Beltran *et al.*,[84] Dardenne *et al.*,[127] Lee *et al.*[128]
EZH2/CBX2 expression	Altered PRC2 methylation activity and H3K27me3 "reading"	AR-independence; NE characteristics; lineage plasticity	Beltran *et al.*,[49] Beltran *et al.*,[84] Martin *et al.*,[123] Bohrer *et al.*,[129] Varambally *et al.*,[130] Clermont *et al.*,[131]
FGF signaling	Activated MAPK pathway; ID1 and BMP expression	Increased cell growth and decreased apoptosis in AR-negative/NE-negative cells	Bluemn *et al.*[95]
DEK gain*	Altered chromatin state; other uncharacterized factors	NE tumor progression	Lin *et al.*[132]

*Further validation needed to truly define DEK as an epigenetic driver. AR: androgen receptor; AURKA: aurora kinase A; BMP: bone morphogenetic protein; BRN2: brain-specific homeobox/POU domain protein 2; CBX2: chromobox homolog 2; DEK: DEK proto-oncogene; EZH2: enhancer of zeste homolog 2; FGF: fibroblast growth factor; FOXA1: forkhead box protein A1; FOXA2: forkhead box protein A2; H3K27me3: histone H3 lysine 27 trimethylation; HES6: hes family bHLH transcription factor 6; HIF-1α: hypoxia-inducible factor 1 alpha subunit; HOXB13: homeobox B13; ID1: inhibitor of DNA binding 1, HLH protein; IL-8: interleukin-8; JMJD1A: jumonji domain-containing 1A; NE: neuroendocrine; NKX3.1: NK3 homeobox 1; MAPK: mitogen-activated protein kinases; MYCN: neuroblastoma-derived v-myc avian myelocytomatosis viral related oncogene; PRC2: polycomb repressive complex 2; PTEN: phosphatase and tensin homolog; RB1: retinoblastoma 1; REST: repressor element (RE)-1 silencing transcription factor; ERK: extracellular signal-regulated kinase; PEG10: Paternally Expressed 10; SOX: sex-determining region Y-box; SRRM4: serine/arginine repetitive matrix 4; TMPRSS2-ERG: transmembrane protease, serine 2-ETS-related gene; TP53: tumor protein p53; UTY: ubiquitously transcribed tetratricopeptide repeat containing, Y-linked; TRAMP: transgenic adenocarcinoma of the mouse prostate

transcription factor SOX2 to AR-independent, therapy-resistant cells.[53] A recent study of dedifferentiated, *PTEN*/*TP53*-altered tumors has revealed that the *TMPRSS2-ERG* gene fusion may restrict the observed lineage plasticity in ERG-positive, *PTEN*/*TP53*-altered tumors.[121] A detailed time-course study of a hormone-naïve patient-derived xenograft model demonstrated that in the context of *RB1*/*TP53* mutation or loss, upregulated paternally expressed 10 (PEG10) promotes increased cell growth in NE-like tumor cells after castration.[89,122] Alternatively, *Pten* and *Trp53* loss is also sufficient to induce prostate tumor cell lineage plasticity in mice, although further orthotopic transplantations of transformed cells resulted in the formation of orthotopic PIN and adenocarcinoma only.[123] Similarly, in a CRPC tumor model with *Pten* and *Trp53* loss and heterozygous loss of *NKX3.1*, transdifferentiation to a NE-like tumor was observed after abiraterone treatment, mediated by neural differentiation factor SOX11.[55] Alternative splicing has also been implicated in transdifferentiation to AR-negative/NE-positive cells. In the context of *RB1* loss, *TP53* loss, or castration, increased expression of the alternative splicing factor SRRM4, which leads to neural-specific exon insertion in genes important for neural cell differentiation, was shown to drive transdifferentiation of LNCaP cells in a xenograft model.[124]

Intriguingly, SOX transcription factors such as SOX2 and SOX11 are highlighted in multiple models and patient cohorts as potential mediators of AR-independent NE-like tumor phenotypes.[53–55,125] Recent study of a LNCaP xenograft model that developed enzalutamide-resistance and a AR-negative/NE-positive tumor phenotype after castration revealed significantly increased levels of brain-specific homeobox/POU domain protein 2 (BRN2), a master regulatory neural transcription factor, as AR levels decreased.[125] *BRN2* expression was shown to be directly repressed by AR. This inverse correlation between BRN2 and AR was also validated in cohorts of patient adenocarcinomas versus CRPCs versus NEPCs.[49,84,125] Additional findings that BRN2 co-regulates neural SOX2 target genes and SOX2 activity suggest BRN2 as a major driver and upstream regulator of the SOX-mediated NE tumor phenotype.[125,126]

In addition to the *RB1*/*PTEN*/*TP53* axis, *MYCN* amplification and *AURKA* amplification have been linked with the loss of AR and progression to poorly differentiated NE tumors, which is also mediated by EZH2, the catalytic subunit of polycomb repressive complex 2 (PRC2) responsible for histone H3 lysine 27 trimethylation (H3K27me3).[84,127,128] EZH2 expression is repressed indirectly by AR[129] and is associated with PCa progression, AR-independent NE-like tumors, and plasticity.[49,84,130] Interestingly, EZH2 and the H3K27me3 chromobox reader chromobox homolog 2 (CBX2) were found to be highly expressed in both a NE xenograft model and NE patient tumors compared to adenocarcinoma.[89,131] Expression of another chromatin modulator, DEK, which induces DNA supercoils and can recruit chromatin remodelers, has also been associated with the transition to AR-independent NE tumors in both a NE xenograft model and patient tumors,[89,132] further highlighting a role for dysregulated epigenetics in prostate tumor reprogramming after hormone therapies.

These studies collectively emphasize a complex network of cooperating genetic and epigenetic alterations that respond to ADT and AR-targeted therapies by initiating plasticity-mediated therapy resistance through master downstream regulators such as *SOX* genes, epigenetic remodelers such as EZH2, and lineage-related transcription factors such as FOXA1 or FOXA2 (**Figure 1**). Further studies to gain a comprehensive understanding of frequently overlapped alterations in patient tumors and the ability to induce transdifferentiation in specific contexts are required.

CONCLUSIONS

Although ADT and antiandrogen therapies are well-established therapeutic options for advanced PCa, unavoidable resistance through mechanisms such as lineage plasticity remains a key barrier. It has become clear that many prevalent PCa-associated alterations can contribute to lineage reprogramming of AR-positive cancer cells after therapy. The precise mutational networks and key regulatory factors that drive plasticity within any tumor represent promising therapeutic targets in combination with hormone therapies. Future efforts must focus on standardizing the molecular and morphological characterizations of pre- and posttherapy tumor subtypes. In addition, the complex regulatory networks that contribute to lineage reprogramming and therapy resistance in the context of each tumor subtype must be elucidated. In particular, a better understanding is needed of the relationship between AR-positive/NE-negative, AR-negative/NE-negative, and AR-negative/NE-positive tumor cells that drive tumor progression after therapy. These studies may reveal new therapeutic targets and combination therapies that prevent the development of further drug resistance.

AUTHOR CONTRIBUTIONS

AMB and HH defined the scope of the review, performed a comprehensive literature search, and wrote the review. Both authors read and approved the final manuscript.

COMPETING INTERESTS

Both authors declared no competing interests.

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