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Neuroendocrine differentiation in prostate cancer: key epigenetic players

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A recent publication by Dardenne *et al.* (1) in *Cancer Cell* (October 10, Vol. 30: 563–577, 2016) demonstrate N-Myc overexpression and its stabilization by Aurora-A (AURKA) and AKT1 Kinase induces enhancer of zeste homolog 2 (EZH2)-mediated transcriptional reprogramming repressing androgen receptor (AR) and driving neuroendocrine prostate cancer (1). This has important clinical implication proposing combinatorial therapy using inhibitors of phosphatidylinositol 3-kinase (PI3K)-AKT and AURKA as future trials in the management of neuroendocrine tumors.

Neuroendocrine differentiation in prostate cancer (NEPC) is frequently associated with advance disease and poor clinical outcome (2,3). The incidence of neuroendocrine phenotypes in primary prostate cancers is approximately 1% whereas in lethal metastatic castrate-resistant prostate cancers its percentage is up to 25–30% (4). Emerging evidence suggests that NEPC develops as a transdifferentiation from prostate adenocarcinoma, in response to androgen deprivation therapy and/or treatment with inhibitors targeting AR signaling pathways (5). During NEPC development, cells lose their granular structure and demonstrate small cell neuroendocrine-like morphology, positive for typical neuroendocrine markers such as chromogranins, synaptophysin (SYP), and neurospecific enolase (NSE) but little or very low levels of AR and AR-regulated gene expression (6). Since AR signaling is required for epithelial cell differentiation during prostate development, inhibition of AR

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Comment on: Dardenne E, Beltran H, Benelli M, *et al.* N-Myc Induces an EZH2-Mediated Transcriptional Program Driving Neuroendocrine Prostate Cancer. *Cancer Cell* 2016;30:563–77.

pathway likely initiates developmental reprogramming of prostate adenocarcinoma to neuroendocrine tumors through a transdifferentiation mechanism (7). Unlike prostate adenocarcinoma, neuroendocrine tumors are very aggressive with median cancer-specific survival is less than 2 years (8). Conventionally, neuroendocrine prostate cancers have been managed clinically with cisplatin-based chemotherapy regimens, however further identification and understanding of the oncogenic drivers of this phenotype necessitates the development of novel targeted therapies.

Dardenne *et al.* (1) performed an integrated analysis employing clinical prostate cancer specimens consisting of NEPC tumor cells and castrate-resistant prostate tumors with focal neuroendocrine differentiation, which correlated higher N-Myc expression in the neuroendocrine phenotype. In parallel studies, authors generated genetically-engineered mouse (GEM) model that harbor a CAG-driven lox-stop-lox human *MYCN* gene integrated into the ROSA26 (LSL-MYCN) locus and a *Tmprss2*-driven tamoxifen-activated Cre recombinase. T2-Cre specifically mediates Cre recombination in luminal cells in the prostate. These mice were cross-bred with other mice that harbor a *Pten* conditional knockout allele leading to increase in PI3K/AKT signaling enhancing N-Myc protein stability. Mice with heterozygous loss of *Pten* and N-Myc overexpression exhibited a divergent mixture of large invasive carcinoma in the prostate with foci containing AR-positive adenocarcinoma or AR-negative NEPC tumor cells, compared with the corresponding control. The NEPC foci displayed high levels of MYCN RNA with N-Myc overexpression associated with enhanced AKT signaling. In another study, Lee *et al.* (9) using a model of premalignant prostatic intraepithelial neoplasia demonstrated that active myristoylated AKT1 together with forced expression of *MYCN* in normal prostate basal cells (but not in luminal cells) led to the development of invasive, metastatic castration-resistant tumors positive for neuroendocrine markers. Furthermore, NEPC tumors with N-Myc overexpression abrogates AR signaling and downregulates AR-target gene *FKBP5*, a member of the immunophilin protein family, which serves as a scaffolding protein for AKT and PHLPP promoting PHLPP dephosphorylation of AKT at amino acid S473 (p-AKT). *FKBP5* downregulation leads to activation of AKT by releasing *FKBP5*-PHLPP-mediated suppression of AKT in the crosstalk between AR signaling and PI3K-AKT pathway. Based on the results, the current study establishes the oncogenic role of MYCN in neuroendocrine prostate cancer. Several published studies have demonstrated the oncogenic role of various genes that likely facilitate the progression of prostate adenocarcinoma to NEPC through AR suppression (5,7), involvement of AR-splice variants (10), and induction of neuroendocrine features and neural programs (11). Many genes associated with a neuroendocrine phenotype, include AR-regulated genes *viz.* *ARG2* (12) and *hASH-1* (13). Additional drivers in the pathogenesis of NEPC include loss of tumor suppressors, activation of mitotic programs, and genomic instability. Tumor suppressors TP53 and RB1 are dysfunctional in various malignancies including NEPC (14,15). The combined deficiency of RB1 and TP53 promotes the transformation to NEPC, as reported in conditional mouse models of NEPC (16). Besides, several cell-cycle genes have been shown to be frequently amplified and/or overexpressed in NEPC, thus supporting their role in driving uncontrolled NEPC growth and proliferation. This list includes UBE2C, cyclin D1, Src family kinase-FYN, and polo-like kinase PLK1 (17–19).

The most noteworthy observation of the study involves binding of N-Myc to AR enhancers and the subsequent interaction of this complex with the members of polycomb repressive complex 2 (PRC2). The polycomb group proteins EZH2, suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED), and RbAp48 form the PRC2 complex, which specifically trimethylates lysine-27 of histone H3 (H3K27) on target gene promoters (20). This histone marker is part of a preprogrammed cellular memory system inherited through mitotic cell divisions and thus preserves cellular identity. Genome-wide location analysis revealed that PRC2 represses a special set of developmental regulators and signaling molecules. Notably, EZH2 is the catalytic subunit of PRC2 and is a highly conserved histone methyltransferase (HMT) that targets H3K27 trimethylation. This trimethylated H3K27 chromatin is commonly associated with silencing of differentiation genes in humans (21). Current study reveals that the catalytic activity of EZH2 facilitates N-Myc/AR/EZH2-PRC2 complex formation. High levels of EZH2 protein and its activity in the prostate of mice overexpressing N-Myc redirects EZH2 activity to N-Myc target gene promoters resulting in transcription repression, whereas EZH2 inhibition reverses N-Myc gene regulation (1). Thus, the interaction between N-Myc, AR, and EZH2 results in the abrogation of AR signaling despite abundant levels of AR, suggesting that the amplification of MYCN and inactivation of AR might be a significant phenomenon in NEPC tumors (Figure 1). Moreover, RE1-silencing transcription factor (REST) is another epigenetic alteration essential in driving the development of the NE phenotype, which is controlled by AR (22). In addition to these epigenetic modifications, several novel somatic mutations in multiple chromatin/histone modifiers including MLL2, UTX and ASXL1 as well as transcription factors FOXA1 and ETS2 were noted in neuroendocrine prostate tumors (23).

Using cell culture and pre-clinical mouse models, the authors further demonstrate that N-Myc forms a complex with AURKA that results in N-Myc stabilization in NEPC tumors. Aurora kinases (A and B), composed of serine/threonine kinase, are essential for cell proliferation. Specifically, AURKA amplification has been reported in over 60% of prostate cancer from patients that developed treatment-related NEPC and in 86% of metastases, whereas concurrent amplification of N-Myc was present in 69% of treated prostate cancer and 83% of metastases (24). In the experiments, pharmacological inhibition of allosteric AURKA inhibitor led to rapid dissociation of the N-Myc-AURKA complex and rapid degradation of N-Myc. In accordance with these findings, Lee *et al.* (9) evaluated the therapeutic efficacy of several AURKA inhibitors and found that CD532, a novel AURKA inhibitor, results in a significant reduction in MYCN protein levels and decreased tumor burden in pre-clinical models driven by MYCN overexpression. Interestingly, this effect was not observed with MLN8237—an AURKA inhibitor, which is currently in early-phase clinical trials, as downregulation of MYCN protein levels did not appear to be dependent on AURKA kinase activity. However, the present study findings demonstrate that N-Myc overexpressing cells exhibit higher sensitivity to AKT inhibitors in combination with allosteric AURKA inhibitor.

Clinical and genomic profiling data propose that neuroendocrine prostate cancers may originate *de novo* from a small population of neuroendocrine cells present in the prostate (2–4). However in the majority of cases these tumors diverge from a population of luminal-derived metastatic castrate-resistant adenocarcinoma. Either way, these two processes are

preferentially mobilized under selective pressure from castration and/or treatment with AR inhibitors in conjunction with genetic perturbations that help initiate or maintain NE phenotype. While the current study establishes the oncogenic role of N-Myc in NEPC, it is still unclear the precise cell of origin of NEPC and their maintenance *in vivo*. It is postulated that the stimulation of growth factor production, such as epidermal growth factor, insulin-like growth factor, keratinocyte growth factor and secretion of some pro-inflammatory cytokines including interleukin: IL-6, IL-8, IL-1 β and macrophage migration inhibitory factor can promote neuroendocrine differentiation through AR pathway in the absence of androgens (25).

The significance of this manuscript lies in its ability to demonstrate the cooperation between N-Myc and EZH2 in repressing AR to drive NEPC. This has important clinical implication and rationale for combining therapy targeting or co-targeting molecules in N-Myc driven tumors through newly designed clinical trials in future.

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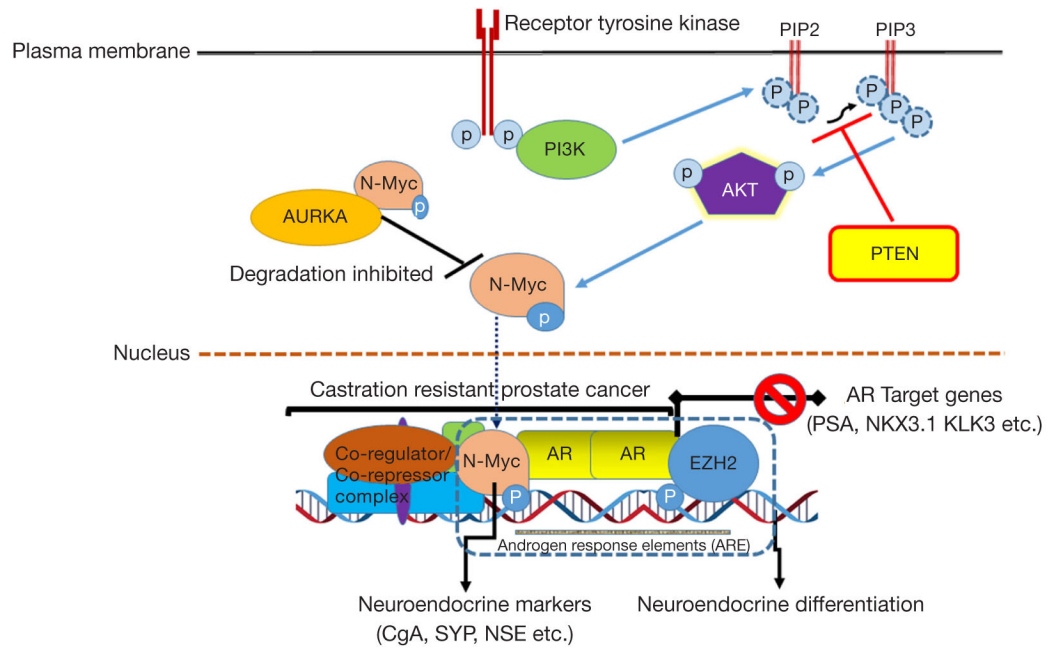


Figure 1.

Schematic model of neuroendocrine differentiation in prostate cancer. N-Myc is overexpressed after first-line or second-line androgen deprivation therapy. AURKA can bind and stabilize N-Myc in the cytosol. In the model, signaling is initiated at the membrane with receptor tyrosine kinases (RTKs) which are activated in ligand-specific manner. RTK activates PI3K through conversion by its catalytic domain of phosphatidylinositol (3,4)-bis-phosphate (PIP2) lipids to phosphatidylinositol (3,4,5)-tris-phosphate (PIP3) where phosphatase and tensin homolog (PTEN), a tumor suppressor lipid phosphatase, converts it back to PIP2. PTEN is frequently mutated or deleted in castrate-resistant prostate cancer and its loss constitutively activates Akt, which in turn phosphorylates N-Myc. Phosphorylated N-Myc translocates to the nucleus, physically binds and forms a complex with EZH2, a catalytic subunit of PRC2 complex and abrogates AR from its binding to androgen-response elements (ARE). The interaction between N-Myc, AR, and EZH2 results in the abrogation of AR signaling, despite abundant levels of AR, represses AR targeted genes *viz.* prostate-specific antigen (PSA), KLK3 and NKX3.1. Notably, MYCN amplification serves an oncogenic driver in driving neuroendocrine differentiation in prostate cancer cells recognized by expression of neuroendocrine markers such as chromogranin A (CgA), SYP, and NSE. AR, androgen receptor; SYP, synaptophysin; NSE, neurospecific enolase.