Targeting neuroendocrine prostate cancer: molecular and clinical perspectives

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INTRODUCTION

For over two decades, several efforts have been made to define and molecularly characterize the frame of neuroendocrine (NE) prostate cancer (NEPC) presenting with distinct clinical features, different from the classic prostatic adenocarcinoma, including frequent visceral metastases, lytic bone involvement, relative low serum prostate-specific antigen (PSA) concentration, resistance to androgen ablation, and high response rate to platinum-based chemotherapy (1). In the era of evolving androgen-directed therapies, better molecular characterization and targeting of the NE phenotype remains of central interest given the inherent or emerging resistance of NEPC cells to current therapies, abrogation of which is warranted in order to improve responses and mortality of prostate cancer (PC) patients.

NEW MORPHOLOGICAL CLASSIFICATION OF NEPC

A new histological classification for NEPC has been recently proposed as a refinement of the current WHO morphologic criteria, to better reflect the aspects of NE differentiation in PC (2). According to the new classification, NEPC extends from usual NE differentiation found in usual prostate adenocarcinoma to mixed (small or large cell) NE carcinoma with acinar adenocarcinoma, adenocarcinoma with Paneth cell NE differentiation, carcinoid tumor, and small cell carcinoma. The new classification also satisfies the previously unmet need of characterizing treatment-induced androgen receptor (AR)-independent PC, which is a significant part of the broader category of castration-resistant tumors clinically defined aggressive-variant, previously known as anaplastic PC (2).

Neuroendocrine prostate carcinoma, either co-present with the local adenocarcinoma disease or as a result of transdifferentiation later in time, was described as one major process of emerging resistance to androgen deprivation therapies, and at the clinical level it is consistent with the development of rapidly progressive visceral disease, often in the absence of elevated serum prostate-specific antigen level. Until present, platinum-based chemotherapy has been the only treatment modality, able to produce a fair amount of responses but of short duration. Recently, several efforts for molecular characterization of this lethal phenotype have resulted in identification of novel signaling factors involved in microenvironment interactions, mitosis, and neural reprogramming as potential therapeutic targets. Ongoing clinical testing of specific inhibitors of these targets, for example, Aurora kinase A inhibitors, in carefully selected patients and exploitation of expression changes of the target before and after manipulation is anticipated to increase the existing data and facilitate therapeutic decision making at this late stage of the disease when hormonal manipulations, even with the newest androgen-directed therapies are no longer feasible.

Keywords: neuroendocrine prostate cancer, small cell prostate carcinoma, targeted therapy, androgen-independent, castration-resistant

CURRENT TREATMENT AND BIOMARKERS

The majority of patients with NEPC are diagnosed with locally advanced disease, usually associated with nodal or/distant metastases. Most often, a cisplatin-based combination (e.g., cisplatin plus etoposide) has been used (3). Although the optimal regimen has not been established, most recent evidence from a phase 2 study of first-line carboplatin and docetaxel (CD) and second-line etoposide and cisplatin (EP) in 120 patients with “anaplastic” PC suggests a high response rate of short duration to platinum-containing chemotherapies, similar to small cell PC. Seventy-four of 113 (65.4%) and 24 of 71 (33.8%) were progression free after four cycles of CD and EP, respectively while median overall survival...
was 16 months. Intriguingly, CEA and LDH emerged as important prognostic indicators in this patient population, in contrast with tissue or serum NE markers including chromogranin A (CgA), synaptophysin, gastrin releasing peptide, somatostatin, which did not predict outcome or response to therapy (4).

Plasma anterior gradient 2 (AGR2) has been proposed as a new promising biomarker for characterization, monitoring, and directing therapies for patients with metastatic NEPC. AGR2 is an epithelial marker regulated by androgens through ErbB3 binding protein 1 and Fox A transcription factors and its circulating tumor cell (CTC) mRNA and serum protein levels were found to be significantly elevated in patients with “anaplastic” cancer (5). Likewise, hASH-1 gene transcription is upregulated by androgen deprivation and is associated to the onset of an NE phenotype (6). However, a recent review of studies on the effect of NE differentiation on oncologic outcomes concludes that data are insufficient to recommend the use of NE markers in routine practice, particularly at early PC stage (7).

EMERGING MOLECULAR TARGETS WITHIN THE LANDSCAPE OF NEPC DIFFERENTIATION

A plethora of different signals involving neuropeptides, growth factors, and cytokines, are involved in the several intracellular processes including angiogenesis, cell survival, proliferation, migration, and invasion through paracrine and autocrine pathways. The resulting changes in more or less all of these processes contribute to the induction of NE differentiation in PC. The model, which has been mostly supported among several others proposed recognizes a role of previous treatment of hormone-naïve PC on the natural evolutionary transformation of classic adenocarcinoma with accumulation of new genetic alterations including loss of tumor suppressors and amplification of onco-genes (8). The finding of rearrangement of TMPRSS2-ERG in NEPC supports the origin of NEPC from prostate adenocarcinoma (8). It was thus suggested that there is an evolutionary continuum from conventional prostate adenocarcinoma to hormone naive state and finally to a CRPC/mixed state as the precursor of NEPC. NEPC has been postulated to correspond to the “cell-autonomous” phase of PC progression, which is the latest evolutionary phase following an “androgen-dependent” and a “microenvironment-driven” phase, respectively. The underlying molecular alterations at this point of progression to NEPC include loss of AR and androgen-regulated protein expression, induction of NE and neural programs, loss of tumor suppressors (TP53, RB1, PTEN) and resultant genomic instability, as well as activation of mitotic programs including Aurora kinase A (AURKA) and Polo-like kinase 1 (PLK1). PLK1 mediates entry into mitosis as well as centromere maturation, spindle checkpoint activity, activation of the anaphase-promoting complex, and eventual exit from the M-phase with the initiation of cytokinesis (13). LNCaP androgen-independent cells were found to have upregulation of the mitotic kinase Plk1 and other M-phase cell-cycle proteins, which rendering them highly sensitive to PLK1 inhibition through necroptosis (14).

ACTIVATION OF MITOSIS

The resultant genetic instability leads to additional changes, many of which affect cell-cycle genes, especially those related to M-phase transition, including AURKA and Polo-like kinase 1 (PLK1). PLK1 mediates entry into mitosis as well as centrosome maturation, spindle checkpoint activity, activation of the anaphase-promoting complex, and eventual exit from the M-phase with the initiation of cytokinesis (13). LNCaP androgen-independent cells were found to have upregulation of the mitotic kinase Plk1 and other M-phase cell-cycle proteins, which rendering them highly sensitive to PLK1 inhibition through necroptosis (14).

AURKA regulates entry into mitosis, as well as assembly of the mitotic spindle apparatus, thereby affecting chromosome separation (15). MYCN amplification is frequently associated with AURKA amplification. In addition, AURKA was found to stabilize MYCN via interaction with the Skp1-Cullin-F-box (SCF)-type ubiquitin ligase FBXW7 that ubiquitinates MYCN and counteracts its degradation (16). C-MYC is also involved in NEPC and was shown to cooperate with the Proto-oncogene serine/threonine-protein kinase 1 (PIM1) in a SCID mice NEPC model, supporting the concept of targeting PIM1 (17). In line with the NEPC mitotic reprogramming, in SCPC/LCNEC xenograft models, high expression of M-phase genes was found, including Ubiquitin-conjugating enzyme E2 C (UBE2C), coupled with RB and cyclin D1 loss, despite the absence of AR expression (18). A sequence of events was therefore suggested in which loss of RB and/or cyclin D1 precede AR loss and further deregulation of the mitotic apparatus.

EPGENETIC REGULATION CHANGES

RE1-silencing transcription factor (REST), also known as neuron-restrictive silencer factor (NRSF) is a transcription factor that represses neuronal differentiation, in NEPC. REST-binding sites were found on 28 of 50 transcriptionally active genes in NEPC and in vivo in a cohort of 218 prostate tumors, in which REST...
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AKT and ERK1/2 signaling pathways. Thus, MIF could represent AR action (28).

Acquisition of endogenous IL-6 production and its possible contribution to an autocrine cell growth stimulation may play an important role during androgen-independent progression (26). IL-6 also participates in a feed-forward loop with pigment epithelium-derived factor (PEDF) to induce NE differentiation, in which NFκB induction elicits STAT3 activation and pro-differentiating IL-6 expression causing further expansion of the NE communications (27). Activation of NFκB pathway is sufficient to maintain androgen-independent growth of prostate PC by regulating AR action (28).

Increased paracrine release of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) during NE differentiation in PC may facilitate cancer progression or recurrence, especially following androgen deprivation, through stimulation of AKT and ERK1/2 signaling pathways. Thus, MIF could represent an attractive target for NEPC therapy (29).

MMP-9 produced by mast cells within the tumor microenvironment enables well-differentiated adenocarcinoma outgrowth by favoring angiogenesis and invasion to the surrounding tissue in TRAMP mice. Upon tumor progression, well-differentiated adenocarcinoma undergoes epithelial-mesenchymal transition (EMT) and foci of poorly differentiated adenocarcinoma are originated. Poorly differentiated adenocarcinoma produces MMP-9 autocrinously, thus becoming independent from mast cells. In the absence of functional mast cells (i.e., when mast cells are inhibited and/or genetically ablated), stem cell factor (SCF) in the prostatic environment is no longer sequestered and becomes available for binding to c-Kit receptor on prostate stem cells. Upon enhanced c-Kit signaling, prostate stem cells continue to proliferate, undergo NE differentiation, and progress to NE tumors, which grow fast and quickly invade the surrounding tissue. The common expression of c-Kit by mast cells and NE clones suggests a possible competition for the ligand SCF and offers the chance of curing early-stage disease while preventing NE tumors using c-Kit–targeted therapy (33, 34).

The implication of EMT in NE transdifferentiation can also occur through the effect of Snail. LNCaP PC cells transfected with Snail displayed increase in the NE markers, neuron-specific enolase (NSE) and CgA, while LNCaP C-33 cells that have been previously reported as a NE differentiation model exhibited increased expression levels of Snail protein as compared with LNCaP parental cells. Functionally, Snail-mediated NE differentiation was associated with increased paracrine cell proliferation. The novel proteasome inhibitor NPI-0052 (salinosporamide A) can inhibit Snail mRNA and protein and thereby promote sensitivity to cisplatin and TRAIL-mediated apoptosis in DU145 PC cells. Natural products including flavonoids and parthenolide have also shown some promise toward targeting Snail signaling in PC (35, 36). Likewise, adrenomedullin, an autocrine/paracrine factor induced by androgen withdrawal, stimulates the NE phenotype in LNCaP prostate tumor cells (37).

Src kinase is another important player in the EMT process as it is activated by several neuropeptides, including CgA, NSE, serotonin, neurophophins, and bombesin. Through direct physical interaction with the AR, Src is able to phosphorylate the AR and thereby induce ligand-independent AR activation (one of the key mechanisms of castration-resistant PC) (38). Neuropeptides, notably bombesin were also found to enhance...
early growth response 1 (Egr-1) expression leading to increased human protease-activated receptor 1 (PAR1) expression and function directly correlating with invasiveness and the degree of PC malignancy (39). Src is also a mediator for the NE-derived parathyroid hormone-related protein (PTHrP), which induces tyrosine phosphorylation and subsequent reduced AR ubiquitination thus increased accumulation of AR, enhancing growth of PC cells at low levels of androgen (40).

In addition, in co-cultures of macrophages with LNCaP and TRAMP-C2 PC cells, a feedback loop between bone morphogenetic protein-6 (BMP-6) derived from PC cells and IL-6 produced by macrophages, resulted in IL-6-induced NE differentiation in PC cells (41).

Receptor activator of nuclear factor kappa-B ligand (RANKL) either derived from the tumor or from the host plays a key role in cancer bone metastasis. A small population of RANKL-expressing cells was observed to initiate and promote cancer bone and soft tissue metastases by recruiting bystander cells to form tumors in bone. The mechanism underlying this recruitment appears to involve a feed-forward mechanism in which RANKL, RANK, and c-Met expression is increased and AR is downregulated. RANKL alters a large transcriptional program that appears to govern formation of the premetastatic niche as well as emergence of osteomimetic, EMT, and stem and NE differentiation (42). When the anti-tumor effects of the bisphosphonate zolendronic acid and somatostatin analogs (SMS) were tested on NE carcinoma models, zolendronic acid, but not SMS induced apoptosis and inhibition of proliferation and migration through impaired prenylation of Ras, thus offering the possibility of therapeutic use in the early phase for controlling NE cells (43).

The concept of "epithelial immune cell-like transition" (EIT), similar to NE-like transdifferentiation of prostate adenocarcinoma cells has been proposed to describe the acquisition of immune properties from cancer cells, which enable them to "communicate" with immune cells, leading to suppression of anti-cancer immune activity in their microenvironment and facilitation of the expansion and malignant progression of the disease (44).

Within this context, a dendritic cell vaccine sipuleucel-T was developed from peripheral blood mononuclear cells obtained by leukapheresis. In randomized trials, sipuleucel-T prolonged overall survival compared with placebo in men with minimally symptomatic, metastatic castrate-resistant PC (CRPC). However, sipuleucel-T did not affect the serum PSA, restricting this approach to patients with slowly progressive disease where a relatively rapid response to treatment is not required (45). In addition, treatment is contraindicated in patients who are on steroids or opioids for cancer-related pain, and should be used with caution in patients with liver metastases. Thus, with so far available data, assessing the impact of immunotherapy on an individual patient with NEPC can be difficult or impossible.

**UPCOMING TARGETED APPROACHES AND PREDICTIVE TOOLS IN NEPC**

In the "cell-autonomous" phase of the disease, inhibitors that affect mitotic function may be efficacious, as opposed to earlier stages when AR signaling affects more "classic" AR-mediated pathways. Currently, first-line treatment for this phase is chemotherapy, but patients become rapidly resistant to this approach. As the molecular basis for NEPC becomes better understood, individualized therapy may be possible.

The AURKA inhibitor danusertib (PHA-739358) was tested in a phase II clinical trial but failed to achieve the primary endpoint of PSA response (46). However, PSA as an endpoint is unlikely to be suitable for tumors that are in the "tumor cell-autonomous" phase. In addition, therapeutic treatment in this trial was not directed specifically to patients with amplified AURKA; hence, it is not certain whether better response would have been achieved by focusing on NEPC patients with amplified AURKA (46). Also, given the enzymatic activity of AURKA depends not only on the amount of protein present but also on the activity of several cofactors (such as TPX2, BORA, and Ajuba), and has numerous substrates (including p53, BRCA1, and even AR), it is likely that the effects of AURKA (and thus the impact of its inhibition) are dependent, at least in part, on the activity of the cofactors and the role of its substrates in a given cell (47). A clinical trial evaluating the AURKA inhibitor alisertib for patients with NEPC is under way, and AURKA and MYCN co-amplification are being explored as potential predictive biomarkers and may be used to select NEPC and patients with high-risk PC for early intervention with AURKA-targeted therapy (48).

Dasatinib is a Src family/abl inhibitor with preclinical activity in PC and encouraging results in phase II studies (49). However, there was no increase in overall survival when dasatinib was given in combination with docetaxel plus prednisone compared with chemotherapy alone. In the phase III READY trial, 1522 men with metastatic CRPC were randomly assigned to either dasatinib with docetaxel plus prednisone or docetaxel plus prednisone alone. With a median follow-up of 19 months, the median survival was approximately 21 months on both treatment arms. This failure can be explained by several factors including inadequate study design, potential pharmacokinetic interactions between dasatinib and docetaxel, a stronger effect on stromal cells than on epithelial cells (despite association with the epithelial-targeted docetaxel), and the too broad specificity of inhibitory effect of dasatinib for numerous receptor and non-receptor tyrosine kinases (50).

PLK1 inhibitors have recently entered clinical trials for solid tumors. BI 2536 is a PLK1 selective inhibitor that reached phase II trial in several solid tumors, but not PC, with little efficacy (51). Volaertib (BI 6727) is a potent and relatively selective inhibitor for PLK1. A Phase I study in patients with advanced disease showed a favorable pharmacokinetic profile and limited toxicities in patients with advanced solid tumors (52). Phase II studies are ongoing. On the basis of the three-phase model, PLK1 inhibitors might be effective in "cell-autonomous" tumors where PLK1 is overexpressed (8).

The role of the tumor microenvironment and data supporting MET as a potential targetable driver of NEPC was also presented as an area of active investigation (53). Cabozantinib (XL184) is an inhibitor of MET, VEGFR2, and RET. A phase II trial in patients with progressive, metastatic PC provided preliminary evidence of activity in men with bone metastases (54). Based upon these results, two phase III trials were initiated in patients who had progressed on docetaxel and either abiraterone or enzalutamide as
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MK-2206 is an oral AKT inhibitor that was tested in a phase 1 trial using a QOD, QW, or Q3W dosing schedule in combination with carboptatin and paclitaxel, docetaxel, or erlotinib, and was well-tolerated at doses that inhibit AKT signaling. Within a diverse population of 72 patients including breast, melanoma, pancreas, prostate, colon, esophageal, small cell lung cancer, a partial response with a PFS of 6 months was shown in a patient with NEPC and minor responses were demonstrated in two patients with NE pancreatic cancers (56). Randomized phase 2 studies in specific cancer types and more homogenous cohorts are expected before being able to draw any conclusions about the clinical effects of MK-2206 with other standard cytotoxic or targeted treatment options.

Given the critical roles of CTCs and EMT in PC tumorigenesis and the current immunotherapeutic strategies targeting prostate tumor antigens, such as sipuleucel-T, there may be a need to design new immunotherapies targeting cancer stem cells and cells involved in EMT.

Cumulative data on currently established and potential future targets of NEPC therapies within corresponding pathways are presented in Table 1. Most evidence for NEPC targets and corresponding targeted agents is derived from preclinical studies or in vivo mouse models (62–68). At the clinical level, there is no direct evidence and all data are extrapolated from studies in CRPC (50, 69–75) (Table 1). Thus, there is an urgent need for exploitation of emerging targets through design and implementation of studies in this particular subpopulation of PC patients with NE differentiated PC.

### CONCLUSIVE REMARKS

The molecular characterization of NEPC is a challenging area of ongoing research with encouraging new findings on potential new targeted therapeutic approaches as well as emerging surrogate biological markers for early identification of treatment responses and failures. However, the determination of appropriate target at the right timepoint within the evolving genotype and phenotype of the disease requires constant reassessment of the underlying molecular changes. Collection and analysis of CTCs offers a great opportunity of repetitively studying these changes as a non-interventional approach compared to tissue biopsy. Ultimately, identification of appropriate targets within the signaling networks that “drive” the evolution of NEPC may not only guide treatment for CRPC. In the COMET-1 trial, patients were randomly assigned to either cabozantinib or prednisone; the primary endpoint was overall survival. In the COMET-2 trial, patients were randomly assigned to cabozantinib or mitoxantrone plus prednisone and the primary endpoint was pain control. Preliminary results from the COMET-1 trial released by the corporate sponsor indicated that cabozantinib did not achieve a statistically significant increase in overall survival (55). Enrollment in the COMET-2 trial was discontinued based upon these results.

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Two oral endothelin receptor antagonists, atrasentan and zibotentan, have been extensively studied, to target the supporting environment for metastatic growth. Multiple phase II and subsequent phase III trials were conducted with both atrasentan and zibotentan; however, none was able to show significant benefit compared with placebo (57–61).

Given the critical roles of CTCs and EMT in PC tumorigenesis and the current immunotherapeutic strategies targeting prostate tumor antigens, such as sipuleucel-T, there may be a need to design new immunotherapies targeting cancer stem cells and cells involved in EMT.

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development of newer biologic treatments but may also enable a more appropriate, in terms of sequencing, utilization of existing therapies to correspond to the underlying molecular biology, which dictates NEPC differentiation.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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