Neuroendocrine Prostate Cancer (NEPC): Are We Selecting For It With Our Current Androgen Annihilation?

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Androgen annihilation as a new therapeutic paradigm in advanced prostate cancer

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• Cardiovascular disease

• Emergence of NEPC
Neuroendocrine cells are part of normal, benign prostate cells

- NEC are typically situated in the basal cell compartment with dendritic cell processes projecting into the layer of luminal.
- Need for specific staining to identify.
- Secrete trophic neuropeptide (bombesin, calcitonin, serotonin, parathyroid like hormone) and growth factors (VEGF).
- Terminally differentiated cells (no proliferative activity, express anti-apoptotic factors).

Primary ("de novo") NEPC

- Very rare (<1%)
- SCCP, large cell NE carcinoma, carcinoid
- Frequent visceral and bulky soft-tissue metastases and limited duration of response to both hormonal therapy and cytotoxic chemotherapy.
- Low serum prostate-specific antigen (PSA) level and high serum levels of NE markers (CgA)
- Treatment involves cisplatin or carboplatin in combination with taxanes
NE differentiation in hormone naïve PC

• 5-10% of prostatic adeno-carcinoma contain clusters/aggregates of “NE like” malignant cells (focal NE differentiation).

• Genetic characterization of these cells suggest their linkage to the neighboring adeno-carcinoma cells.

• Unclear prognostic significance.
Secondary development of NEPC

• Common, estimated to represent up to 25% of lethal prostate cancer.
• Trans-differentiation of adeno-carcinoma cells (epithelial plasticity d/t selective pressure?, common progenitor?).
• Resistant to ADT
• May promote Adeno-carcinoma cell tumorigenicity through paracrine non AR mediated pathways

Terry and Beltran 2014
NE differentiation is very common in metastatic sites

- Evaluation of an archival set of metastatic site biopsies (MSB) to determine NED expression patterns
- 237 MSB from 187 pts. bone (102), lung (40), liver (40), lymph node (20), bladder (14), soft tissue (11), brain (4), others (4).
- IHC for chromogranin-A and synaptophysin.
- All tumors were adenocarcinomas or poorly differentiated carcinomas
- No small cell carcinoma found, BUT, 50% showed positive NED.
- NED expression was positive in 41% bone sites, compared to 53% of non-bone sites
- **NED expression was observed in 44% of hormone sensitive cases and 56% of CRPC cases.**

*Jimenez et al ASCO meeting 2014*

CTCs from patients with NEPC identified in a subset (10.7%) of CRPC patients.

*Beltran et al, Clinic Cancer Res 2015*
Trans-differentiation from epithelial-like phenotype to a NE-like phenotype as a consequence of treatment induced-selective pressure?
Modeling *in-vitro*

- LNCaP cells
- Androgen depletion induce NE-differentiation
- Restoring androgens suppress NE-differentiation

Acquisition of an NE phenotype by PCa cells can be induced by chronic exposure to docetaxel

*Terry et al, Neoplasia 2013*
The trans differentiation process from epithelial to neuroendocrine tumor phenotype can be considered a consequence of the selective pressure (ADT)

- NEC lack the AR
- NEC are deficient in cell regulators (P53, RB1)
- NEC over-express cell cycle genes (ex. cyclin D1, AURA Kinase A- AURKA)
- Androgen receptors splice variants are associated with up regulation of NE genes (AGR2, AURKA, SSTR2) – *Ferrari ASCO 2014 meeting*
Over-expression of protocadherin-PC (PCDH-PC or PCDH11Y) can drive NE trans-differentiation

- ADT upregulates PCDH-PC
- PCDH-PC is an anti-apoptotic gene.
- Encodes on the Y-chromosome (Yp11.2)
- PCDH-PC expression reflects early-onset adaptive mechanism following ADT
- PCDH-PC over-expression induces NE phenotype in PC cells and promotes their survival under diverse stress conditions.

Terry et al, Neoplasia 2013
Is the plasticity induced by the selective pressure reversible?

• In patients with \textit{EGFR}-mutant non–small-cell lung cancer who develop small-cell features as a mechanism of resistance to EGFR inhibition, the discontinuation of the EGFR inhibitor results in reversal of the small-cell phenotype.

• It is not known whether clinically such plasticity exists in small-cell/neuroendocrine prostate cancer.

\textit{Sequist LV et al. Sci Transl Med. 2011.}
Molecular pathways that are involved in NE differentiation of prostate cancer

- RB loss
- MYC over-expression
- PCDH-PC upregulation
- AURKA over-expression
- FoxA2/HIF-1α complex
- Down-regulation of RE1-silencer factor (REST)
Androgens inhibits REST expression which regulates NE differentiation in LNCaP cells.
A common progenitor cell?
FVB-TRAMP mice have worse survival than B6-TRAMP mice because of rapid development and progression of NEC-PC arising from bi-potential stem cells that express epithelial (E-cadherin) and NE (synaptophysin) markers and FOX1/FOX2 transcription factors.

"Chiaverotti et al The American Journal of Pathology 2008"
**ERG gene rearrangements are common in prostatic small cell carcinomas**

- The occurrence of ERG gene rearrangements was examined by fluorescence in situ hybridization in prostatic, bladder and lung small cell carcinomas.

- Presence of ERG rearrangements was found in nearly half of the prostatic small cell carcinomas is a similar rate of rearrangement to that found in prostatic acinar carcinomas.

- No cases of bladder or lung small cell carcinomas showed ERG rearrangement.

- A high concordance rate of ERG rearrangement between the small cell and acinar components in a given patient was found (83%).

- **These findings support a common origin for acinar prostatic adenocarcinoma and small cell carcinoma of the prostate**

*Lotan et al, Mod Pathol 2011*
WISH-PC2: A Unique Xenograft Model of Human Prostatic Small Cell Carcinoma

Androgen responsive growth of pure SSCP is related to off target growth effects of testosterone

• in the first generation, 20% of the mice into which the tumor pieces were implanted had elevated serum PSA levels

• The WISH-PC2 xenograft grows relatively rapidly and with a high take rate (90–100% of the animals).

• Androgens enhance the growth of the AR-negative xenograft, probably via an indirect effect on the surrounding stroma.

<table>
<thead>
<tr>
<th>Table 1 Phenotypic features of WISH-PC2</th>
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<tbody>
<tr>
<td>Feature</td>
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<tr>
<td>DNA ploidy</td>
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<tr>
<td>Proliferative Index (Ki-67)</td>
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<tr>
<td>Bcl-2</td>
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<tr>
<td>Mutated p53</td>
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<td>MDR1 gene product</td>
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<tr>
<td>Her-4/ncu</td>
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<td>MHC Class-I</td>
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\*a Determined by immunohistochemistry.
\*b Determined by RT-PCR.
\*c Determined by Western blot analysis.
\*d Determined by ELISA of murine host plasma.
\*e Determined by FACS analysis.
NE cells can function as endocrine-paracrine cells of the prostate

• A suggested role as intraepithelial regulatory cells displaying hybrid epithelial, neural, and endocrine characteristics.

• secreting alternative growth factors such as bombesin, serotonin, somatostatin, calcitonin, and parathyroid hormone-related protein.

• SCCP is composed of an enriched population of androgen-independent cells whose growth is sustained through alternate paracrine and autocrine pathways
Aurora Kinase A is over-expressed (gene amplification) in NEPC

Inhibition of AURKA (PHA-730358) suppresses the growth of NEPC
Aurora Kinase A Inhibitor MLN8237

Treatment

- Orally administered Aurora kinase A inhibitor.

- 50 mg twice daily for 7 days repeated every 21 days.

- Multi-institutional single-arm, open-label Phase 2 trial in patients with metastatic castrate resistant and NEPC (SCPC, adenocarcinoma plus > 50% immunohistochemical staining for NE markers. Response and progression (primary end point) are evaluated by CT/MRI scan and bone scan after every 3 cycles.

Prevention?

- AURKA amplification in primary adenocarcinoma of the prostate predicts for late stage development of NEPC in CRPC patients.

(Terry and Beltran 2014)
Take home massages (cognitive doggy bag)

- NEPC develops from adeno-carcinoma of the prostate
- This process is a result selective pressure (ADT and cytotoxic agents) and involves specific molecular pathways (PCDH-PC, AURAKA, REST)
- An increase incidence of NEPC in the current era of novel ADT agents is suspected.
- NEPC cells secret alternative growth factors such and thus can promote AR independent growth.
- Potential for targeted therapy (Aurora kinase A inhibitors)